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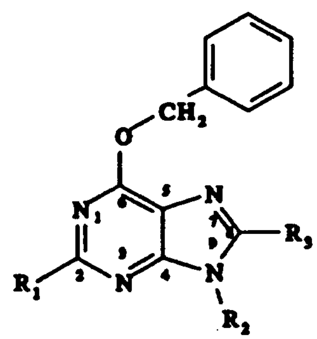
(54) Title: SUBSTITUTED O^6-BENZYLGUANINES AND 6(4)-BENZYLOXYPYRIMIDINES

(57) Abstract



(I)

The present invention provides AGT inactivating compounds such as substituted O^6-benzylguanines of formula (I) wherein 7- or 9-substituted 8-aza-O^6-benzylguanines, 7,8-disubstituted O^6-benzylguanines, 7,9-disubstituted O^6-benzylguanines, 4(6)-substituted 2-amino-5-nitro-6(4)-benzyloxypyrimidines, and 4(6)-substituted 2-amino-5-nitroso-6(4)-benzyloxypyrimidines, as well as pharmaceutical compositions comprising such compounds along with a pharmaceutically acceptable carrier. The present invention further provides a method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent which causes cytotoxic lesion at the O^6-position of guanine, by administering to a mammal an effective amount of one of the aforesaid compounds, 2,4-diamino-6-benzyloxy-*s*-triazine, 5-substituted 2,4-diamino-6-benzyloxypyrimidines, or 8-aza-O^6-benzylguanine, and administering to the mammal an effective amount of an antineoplastic alkylating agent which causes cytotoxic lesions of the O^6-position of guanine.

SUBSTITUTED O^6-BENZYLGUANINES AND 6(4)-BENZYLOXYPYRIMIDINES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to substituted O^6-benzylguanines, O^6-benzyl-8-azaguanines, and 6(4)-benzyloxypyrimidines, pharmaceutical compositions comprising such compounds, and methods of using such compounds. The subject compounds are particularly useful in inactivating the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase.

BACKGROUND OF THE INVENTION

The inactivation of the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase (AGT) by O^6-benzylguanine leads to a dramatic enhancement in the cytotoxic response of human tumor cells and tumor xenografts to chemotherapeutic drugs whose mechanism of action involves modification of DNA guanine residues at the O^6-position (Dolan et al., *Proc. Natl. Acad. Sci. U.S.A.*, *87*, 5368-5372 (1990); Dolan et al., *Cancer Res.*, *51*, 3367-3372 (1991); Dolan et al., *Cancer Commun.*, *2*, 371-377 (1990); Mitchell et al., *Cancer Res.*, *52*, 1171-1175 (1992); Friedman et al., *J. Natl. Cancer Inst.*, *84*, 1926-1931 (1992); Felker et al., *Cancer Chem. Pharmacol.*, *32*, 471-476 (1993); Dolan et al., *Cancer Chem. Pharmacol.*, *32*, 221-225 (1993); Dolan et al., *Biochem. Pharmacol.*, *46*, 285-290 (1993)). The AGT inactivating activity of a large number of O^6-benzylguanine analogs have been compared with the aim of obtaining information about the types of substituent groups and the sites at which they could be attached to O^6-benzylguanine without significantly lowering its AGT-inactivating activity (Moschel et al., *J. Med. Chem.*, *35*, 4486-4491 (1992); Chae et al., *J. Med. Chem.*, *37*, 342-347 (1994)). While these studies led to the production of a variety of analogs that were as potent or somewhat less potent than O^6-benzylguanine, none of the analogs were better than O^6-benzylguanine.

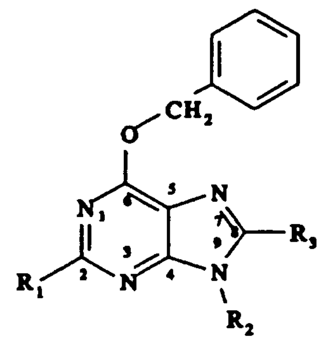
Thus, there remains a need for additional compounds which are capable of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine. The present invention provides such compounds and associated pharmaceutical compositions and treatment methods. These and other objects and advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides 7- and 8-substituted O^6-benzylguanine derivatives, 7,8-disubstituted O^6-benzylguanine derivatives, 7,9-disubstituted O^6-benzylguanine derivatives, 8-aza-O^6-benzylguanine derivatives, and 4(6)-substituted 2-amino-5-nitro-6(4)-benzyloxypyrimidine and 2-amino-5-nitroso-6(4)-benzyloxypyrimidine derivatives which have been found to be effective AGT inactivators, as well as pharmaceutical compositions comprising such derivatives along with a pharmaceutically acceptable carrier. The present invention further provides a method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine, by administering to a mammal an effective amount of one of the aforesaid derivatives, 2,4-diamino-6-benzyloxy-*s*-triazine, 5-substituted 2,4-diamino-6-benzyloxypyrimidines, or 8-aza-O^6-benzylguanine, and administering to the mammal an effective amount of an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a compound of the formula I



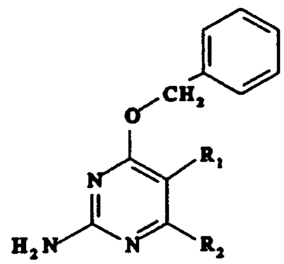
(I)

wherein R_1 is a substituent selected from the group consisting of amino, hydroxy, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, and C_1-C_4 acylamino (although, as explained in further detail below, other substituents can be placed at this 2-position), R_2 is a substituent selected from the group consisting of hydrogen, C_1-C_4 alkyl, C_1-C_4 aminoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkylaminoalkyl, C_1-C_4 dialkylaminoalkyl, C_1-C_4 cyanoalkyl, C_1-C_4 carbamoylalkyl, C_1-C_4 pivaloylalkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, carbo C_1-C_4 alkoxyalkyl, ribose, 2'-deoxyribose, the conjugate acid form of a C_1-C_4 carboxyalkyl, and the carboxylate anion of a C_1-C_4 carboxyalkyl as the sodium salt (although, as explained in further detail below, other substituents can be placed at this N^9-position), and R_3 is a substituent selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, thiol, C_1-C_4 alkylthio, trifluoromethylthio, C_1-C_4 thioacyl, hydroxy, C_1-C_4 alkoxy, trifluoromethoxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, C_1-C_4 acyloxy, amino, C_1-C_4 aminoalkyl, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, trifluoromethylamino, ditrifluoromethylamino, aminomethanesulfonyl, C_1-C_4 aminoacyl, aminotrifluoromethylcarbonyl, formylamino, nitro, nitroso, C_1-C_4 alkyldiazo, C_5-C_6 aryldiazo, trifluoromethyl, C_1-C_4 haloalkyl, halomethyl, C_1-C_4 cyanoalkyl, cyanomethyl, cyano,

C_1-C_4 alkyloxycarbonyl, C_1-C_4 alkylcarbonyl, phenyl, phenylcarbonyl, formyl, C_1-C_4 alkoxymethyl, phenoxymethyl, C_2-C_4 vinyl, C_2-C_4 ethynyl, and SO_nR' wherein n is 0, 1, 2, or 3 and R' is hydrogen, C_1-C_4 alkyl, amino, or phenyl, with the proviso that R_1 is not amino when both R_2 and R_3 are hydrogen, and with the proviso that R_1 is not amino or methylamino when R_2 is ribose or 2'-deoxyribose and R_3 is hydrogen. It is to be understood that the substituents are defined herein such that the group farthest from the point of attachment of the substituent is named first. By way of illustration, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl includes pivaloyloxymethyl.

Suitable compounds of the above formula include those compounds wherein R_1 is selected from the group consisting of amino, hydroxy, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, and C_1-C_4 alkylcarbonylamino, R_2 is selected from the group consisting of hydrogen, C_1-C_4 alkyl, and C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, and R_3 is selected from the group consisting of amino, halo, C_1-C_4 alkyl, hydroxy, and trifluoromethyl. Other suitable compounds include those wherein R_1 is selected from the group consisting of amino, hydroxy, methylamino, dimethylamino, and acetylamino, R_2 is selected from the group consisting of hydrogen, methyl, and pivaloyloxymethyl, and R_3 is selected from the group consisting of amino, bromo, methyl, hydroxy, and trifluoromethyl. Examples of suitable compounds include 8-amino-O^6-benzylguanine, 8-methyl-O^6-benzylguanine, 8-hydroxy-O^6-benzylguanine, 8-bromo-O^6-benzylguanine, 8-trifluoromethyl-O^6-benzylguanine, O^6-benzylxanthine, O^6-benzyluric acid, N^2-acetyl-O^6-benzyl-8-oxoguanine, O^6-benzyl-N^2-methylguanine, O^6-benzyl-N^2,N^2-dimethylguanine, O^6-benzyl-8-trifluoromethyl-9-methylguanine, O^6-benzyl-8-bromo-9-methylguanine, and O^6-benzyl-8-bromo-9-(pivaloyloxymethyl)guanine.

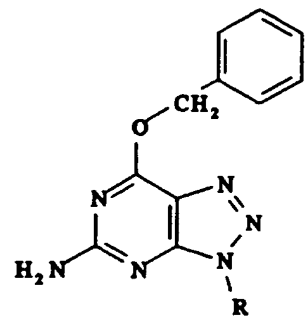
The present invention also provides a compound of the formula II



(II)

wherein R_1 is NO_2 or NO, and R_2 is a substituent selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, thiol, C_1-C_4 alkylthio, trifluoromethylthio, C_1-C_4 thioacyl, hydroxy, C_1-C_4 alkyloxy, trifluoromethoxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, C_1-C_4 acyloxy, C_1-C_4 aminoalkyl, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, trifluoromethylamino, ditrifluoromethylamino, aminomethanesulfonyl, amino C_1-C_4 alkylcarbonyl, aminotrifluoromethylcarbonyl, formylamino, nitro, nitroso, C_1-C_4 alkyldiazo, C_5-C_6 aryldiazo, trifluoromethyl, C_1-C_4 haloalkyl, cyanomethyl, C_1-C_4 cyanoalkyl, cyano, C_1-C_4 alkyloxycarbonyl, C_1-C_4 alkylcarbonyl, phenyl, phenylcarbonyl, formyl, C_1-C_4 alkoxymethyl, phenoxymethyl, C_2-C_4 vinyl, C_2-C_4 ethynyl, and SO_nR' wherein n is 0, 1, 2, or 3 and R' is hydrogen, C_1-C_4 alkyl, amino, or phenyl. Suitable compounds include those compounds wherein R_1 is NO_2 and R_2 is hydrogen or a C_1-C_4 alkyl. Examples of suitable compounds include 2-amino-4-benzyloxy-5-nitropyrimidine and 2-amino-4-benzyloxy-6-methyl-5-nitropyrimidine.

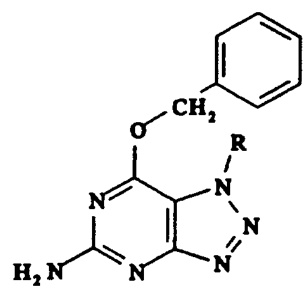
The present invention further provides a compound of the formula III



(III)

wherein R is selected from the group consisting of C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl. Suitable compounds of the above formula include those wherein R is selected from the group consisting of C_1-C_4 alkyl and C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl. Examples of suitable compounds include 8-aza-O^6-benzyl-9-methylguanine and 8-aza-O^6-benzyl-9-(pivaloyloxymethyl)guanine.

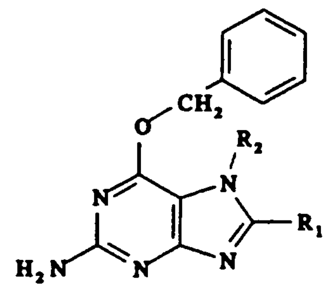
The present invention further provides a compound of the formula IV



(IV)

wherein R is selected from the group consisting of C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl. Suitable compounds include those wherein said R is C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl. An example of a suitable compound is 8-aza-O^6-benzyl-7-(pivaloyloxymethyl)guanine.

The present invention further provides a compound of the formula V



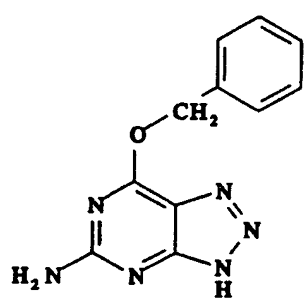
(V)

wherein R_1 is selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, halo C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl, and R_2 is selectd from the group consisting of C_1-C_4 alkyl, halo C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl, with the proviso that when R_1 is hydrogen, R_2 is selected from the group consisting of halo C_1-C_4 alkyl, C_1-C_4 alkyloxy C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_3-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_2-C_4 alkyl, cyano C_2-C_4 alkyl, aminocarbonyl C_2-C_4 alkyl, and hydroxy C_1-C_3 alkyl. Suitable compounds include those wherein R_1 is hydrogen or halo, and R_2 is C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl. Examples of suitable compounds include O^6-benzyl-8-bromo-7-(pivaloyloxymethyl)guanine and O^6-benzyl-7-(pivaloyloxymethyl)guanine.

The present invention additionally provides treatment methods, which are generally administered via pharmaceutical compositions comprising one or more of the O^6-substituted compounds of the present invention. In particular, the present invention provides a method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent that

causes cytotoxic lesions at the O^6-position of guanine, which method comprises administering to a mammal an effective amount of one or more of the aforedescribed present inventive compounds of formulas I-V, and administering to the mammal an effective amount of an antineoplastic alkylating agent that causes cytotoxic lesions at the O^6-position of guanine. The present invention also includes the method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent that causes cytotoxic lesions at the O^6-position of guanine, which method comprises (i) administering to a mammal an effective amount of

(a) 8-aza-O^6-benzylguanine



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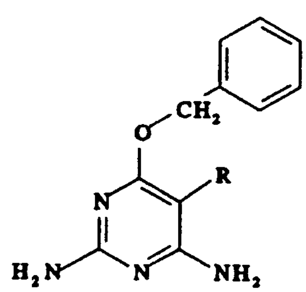
(b) a compound of the formula VI



(VI)

wherein R is a substituent selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, thiol, C_1-C_4 alkylthio, trifluoromethylthio, C_1-C_4 thioacyl, hydroxy, C_1-C_4 alkoxy, trifluoromethoxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, C_1-C_4 acyloxy, amino, C_1-C_4 aminoalkyl, C_1-C_4 alkylamino, C_1-C_4

dialkylamino, trifluoromethylamino, ditrifluoromethylamino, aminomethanesulfonyl, amino C_1-C_4 alkylcarbonyl, aminotrifluoromethylcarbonyl, formylamino, nitro, nitroso, C_1-C_4 alkyldiazo, C_5-C_6 aryldiazo, trifluoromethyl, C_1-C_4 haloalkyl, halomethyl, cyanomethyl, C_1-C_4 cyanoalkyl, cyano, C_1-C_4 alkyloxycarbonyl, C_1-C_4 alkylcarbonyl, phenyl, phenylcarbonyl, formyl, C_1-C_4 alkoxymethyl, phenoxymethyl, C_2-C_4 vinyl, C_2-C_4 ethynyl, and SO_nR' wherein n is 0, 1, 2, or 3 and R' is hydrogen, C_1-C_4 alkyl, amino, or phenyl, or

(c) 2,4-diamino-O^6-benzyl-*s*-triazine,

and (ii) administering to the mammal an effective amount of an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine.

Various substitutions onto the present inventive compounds and those compounds useful in the context of the present inventive method are possible while retaining the effectiveness of those compounds. In particular, the N^9 position of the compounds of formula I and 8-aza-O^6-benzylguanine, and the 2- and/or 4-positions of the compounds of formulas I-VI, can be substituted with a variety of substituents (which can be used instead of any existing substituents at those positions). Such substitutions include aryl, a substituted aryl wherein the aryl substituents are selected from the group consisting of C_1-C_6 alkyl, C_1-C_6 alkoxy, nitro, halo, a polycyclic aromatic alkyl containing 2-4 aromatic rings, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 hydroxyalkyl, C_1-C_6 halohydroxy alkyl, C_1-C_6 alkylcarbonyloxy, alkylcarbonyloxyalkyl wherein the alkyl is C_1-C_6, carboxy, the acid or salt form of carboxyalkyl wherein the alkyl is C_1-C_6, carbonyl, carbamoyl, a carbamoylalkyl wherein the alkyl is C_1-C_6, hydrazinocarbonyl, chlorocarbonyl, cyano, C_2-C_9 cyanoalkyl, C-formyl, a dialkoxymethyl wherein the alkoxy is C_1-C_6, C-alkylcarbonyl, an alkoxy hydroxyalkyl wherein the alkyl and the alkoxy are C_1-C_6, carboxymethyl thio, a carboalkoxy alkyl wherein the alkoxy and alkyl are C_1-C_6, a monoalkylamino hydroxylalkyl wherein the alkyl is

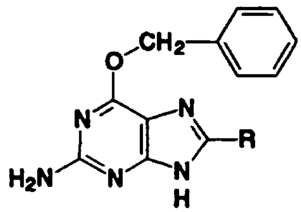
C_1-C_6, a dialkylamino hydroxyalkyl wherein the alkyl is C_1-C_6, amino hydroxyalkyl wherein the alkyl is C_1-C_6, a peptide derived from the β-lactone of L-serine, or related amino acids, a monosaccharide selected from the group consisting of aldotetroses, aldopentoses and aldohexoses, a polysaccharide selected from the group consisting of sucrose, lactose, maltose and cellobiose, a nucleic acid segment, a steroid selected from the group consisting of testosterone, nortestosterone, and dihydrotestosterone, and SO_nR' wherein n is 0, 1, 2, or 3 and R' is H, C_1-C_6 alkyl or aryl.

The benzene ring of the benzyl groups also may be substituted with one or more suitable substituents such as, for example, hydrogen, halo, nitro, nitroso, aryl, substituted aryl, aralkyl, substituted aralkyl, polycyclic aromatic arylalkyl, alkyl, cycloalkyl, alkenyl, alkynyl, trifluoromethyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, aryloxy, alkylcarbonyloxy, alkylcarbonyloxyalkyl, oxo, amino, monoalkylamino, dialkylamino, hydrazino, hydroxyamino, acylamino, ureido, thioureido, amidino, guanidino, carboxy, carboxyalkyl, alkoxycarbonyl, carbamoyl, hydrazinocarbonyl, chlorocarbonyl, cyano, cyanoalkyl, C-formyl, dialkoxymethyl, C-alkylcarbonyl, cyanato, isocyanato, thiocyanato, isothiocyanato, carboxymethylthio, aminoalkyl, alkylamino, aminocarboxyalkyl, peptides, carbohydrate, polysaccharide, steroid, heterocycle, aromatic heterocycle, nucleic acid derivative, and SO_nR_1 wherein n is 0, 1, 2, or 3, and R_1 is hydrogen, alkyl or aryl. The hydrocarbon substituents, such as alkyl, alkoxy, aminoalkyl, alkylamino, and the like, will typically be C_1-C_6, more typically C_1-C_4.

The range of substituents found useful with respect to the identified positions on the compounds of formulas I-VI are generally electron withdrawing, as noted in applicants' related U.S. patent application Serial No. 08/255,190 and hereinbelow. Further information regarding useful

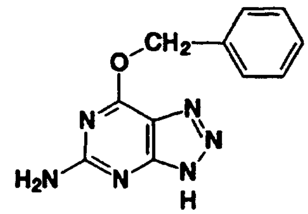
substituents for AGT depleting compounds of the present invention is also provided in U.S. Patents 5,091,430, 5,358,952, and 5,352,669. Collectively, the aforedescribed compounds useful in the context of the present invention are referred to herein as O^6-substituted compounds.

Several O^6-substituted compounds were tested for their ability to inactivate the human DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT, alkyltransferase). Two classes of compounds were identified as being significantly better than O^6-benzylguanine (the prototype low-molecular-weight inactivator) in inactivating AGT in human HT29 colon tumor cell extracts. These were 8-substituted O^6-benzylguanines bearing electron-withdrawing groups at the 8-position and 5-substituted 2,4-diamino-6-benzyloxypyrimidines bearing electron-withdrawing groups at the 5-position. The latter derivatives were also more effective than O^6-benzylguanine in inactivating AGT in intact HT29 colon tumor cells. Both types of compounds were as effective or more effective than O^6-benzylguanine in enhancing cell killing by 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU) of colon, breast and prostate cancer cells grown in culture. Provided 8-substituted O^6-benzylguanine derivatives bearing electron-withdrawing substituents at the 8-position and 5-substituted 2,4-diamino-6-benzyloxypyrimidines bearing electron-withdrawing substituents at the 5-position do not exhibit undesirable toxicity, they should be superior to O^6-benzylguanine as chemotherapeutic adjuvants for enhancing the effectiveness of antitumor drugs whose mechanism of action involves modification of the O^6-position of DNA guanine residues. The specific compounds surveyed for AGT inactivating activity are illustrated below.



1a-e

1a, R=NH_2
1b, R=CH_3
1c, R=OH
1d, R=Br
1e, R=CF_3



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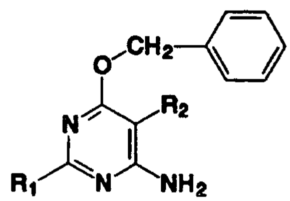
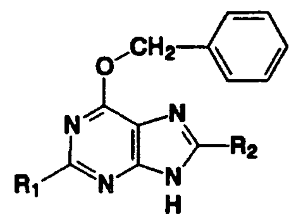


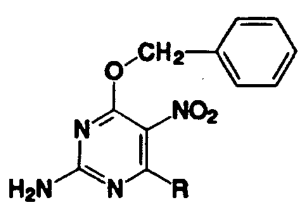
3a-f

3a, R_1=H; R_2=NO_2
3b, R_1=NH_2; R_2=H
3c, R_1=NH_2; R_2=NH_2
3d, R_1=NH_2; R_2=NO
3e, R_1=NH_2; R_2=NO_2
3f, R_1=NH_2; R_2=Br



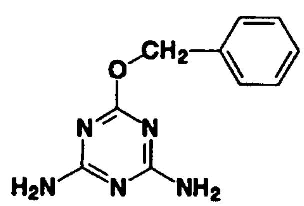
4a-f

4a, R_1=HO; R_2=H
4b, R_1=HO; R_2=OH
4c, R_1=F; R_2=H
4d, R_1=CH_3CONH; R_2=OH
4e, R_1=CH_3NH; R_2=H
4f, R_1=$(CH_3)_2N$; R_2=H



5a,b

5a, R = H
5b, R = CH_3



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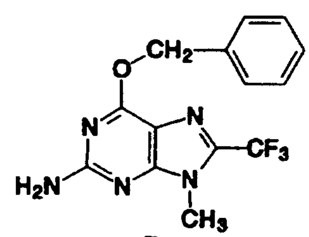

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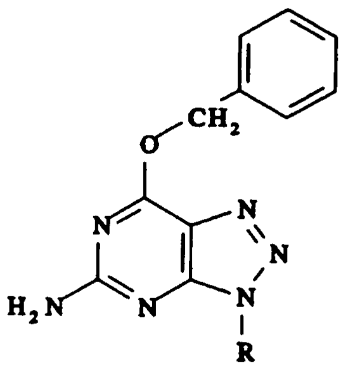
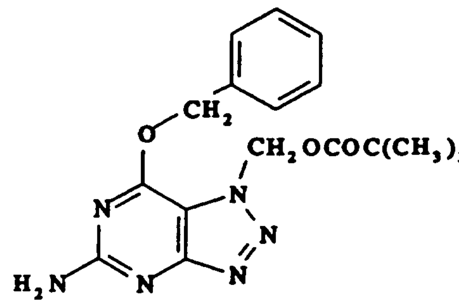


8a-b

8a, R = CH_3
8b, R = $CH_2OCOC(CH_3)_3$



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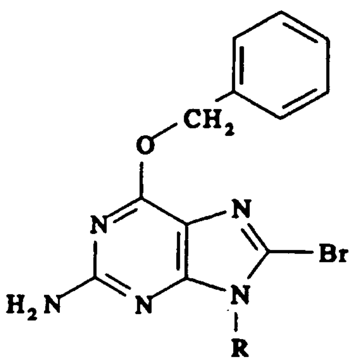
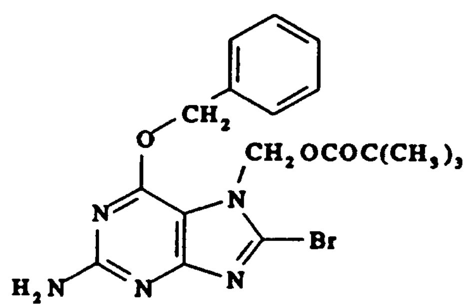


10a-b

10a, R = CH_3
10b, R = $CH_2OCOC(CH_3)_3$



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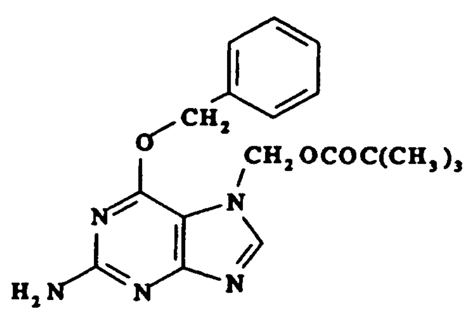


12

Preparations of the 8-substituted O^6-benzylguanine derivatives 8-amino-O^6-benzylguanine (**1a**) and O^6-benzyl-8-methylguanine (**1b**) were accomplished by treating 2,8-diamino-6-chloropurine and 2-amino-6-chloro-8-methylpurine, respectively, with sodium benzyloxide in benzyl alcohol. O^6-Benzyl-8-oxoguanine (O^6-benzyl-7,8-dihydro-8-oxoguanine, **1c**) was prepared by reacting 1,1'-carbonyldiimidazole with 2,4,5-triamino-6-benzyloxypyrimidine (Pfleiderer et al., *Chem. Ber.*, *94*, 12-18 (1961)). For convenience, the compound is illustrated in the 8-hydroxy tautomeric form although it most probably exists in solution in the 8-keto form with a hydrogen attached to the 7-nitrogen atom. O^6-Benzyl-8-bromoguanine (**1d**) was prepared by bromination of O^6-benzylguanine. O^6-Benzyl-8-trifluoromethylguanine (**1e**) was prepared by reacting 2-amino-6-chloro-8-trifluoromethylpurine with sodium benzyloxide in benzyl alcohol. 8-Aza-O^6-benzylguanine (**2**) was prepared through nitrous acid treatment of 2,4,5-triamino-6-benzyloxypyrimidine. Compound **2** had been prepared previously by another route (Shealy et al., *J. Org. Chem.*, *27*, 4518-4523 (1962)).

With respect to the pyrimidine derivatives (**3a-f**), 4-amino-6-benzyloxy-5-nitropyrimidine (**3a**) was prepared by treating 4-amino-6-chloro-5-nitropyrimidine (Boon et al., *J. Chem. Soc.*, 96-102 (1951)) with sodium benzyloxide in benzyl alcohol. Derivatives **3b-d** were prepared by the method of Pfleiderer et al. (*Chem. Ber.*, *94*, 12-18 (1961)). 2,4-Diamino-6-benzyloxy-5-nitropyrimidine (**3e**) and 2,4-diamino-6-benzyloxy-5-bromopyrimidine (**3f**) were prepared previously by Kosary et al. (*Acta Pharm. Hung.*, *49*, 241-247 (1989)).

The purines, O^6-benzylxanthine (**4a**) and O^6-benzyluric acid (**4b**) were prepared by nitrous acid deamination of O^6-benzylguanine and O^6-benzyl-8-oxoguanine, respectively. N^2-Acetyl-O^6-benzyl-8-oxoguanine (N^2-acetyl-O^6-benzyl-7,8-dihydro-8-oxoguanine) (**4d**) was prepared through acetylation of O^6-benzyl-8-oxoguanine (**1c**). O^6-Benzyl-2-fluorohypoxanthine (**4c**) was prepared previously by Robins

and Robins (*J. Org. Chem.*, *34*, 2160-2163 (1969)). This material was treated with methylamine and dimethylamine to produce O^6-benzyl-N^2-methylguanine (**4e**) and O^6-benzyl-N^2,N^2-dimethylguanine (**4f**), respectively.

Compounds **5a** (2-amino-4-benzyloxy-5-nitropyrimidine) and **5b** (2-amino-4-benzyloxy-6-methyl-5-nitropyrimidine) were prepared by treating 2-amino-4-chloro-5-nitropyrimidine and 2-amino-4-chloro-6-methyl-5-nitropyrimidine (Boon et al., *J. Chem. Soc.*, 96-102 (1951)), respectively, with sodium benzyloxide in benzyl alcohol. Compound **6** (2,4-diamino-6-benzyloxy-*s*-triazine) was prepared previously under similar conditions (Wakabayashi et al., *Nippon Dojo-Hiryyogaku Zasshi*, *41*, 193-200 (1970)). O^6-Benzyl-8-trifluoromethyl-9-methylguanine (**7**) was prepared by treating the anion of **1e** with methyl iodide in *N,N*-dimethylformamide.

Compound **8a** was prepared by methylating the sodium salt of 8-aza-O^6-benzylguanine using methyl iodide as the methylating agent. Compounds **8b** and **9** were prepared by the reaction of the sodium salt of 8-aza-O^6-benzylguanine and chloromethyl pivalate. Compound **10a** was prepared by direct bromination of O^6-benzyl-9-methylguanine. Compounds **10b** and **11** were prepared by the reaction of the sodium salt of O^6-benzyl-8-bromoguanine and chloromethyl pivalate. Compound **12** was prepared by the reaction of the sodium salt of O^6-benzylguanine and chloromethyl pivalate.

The ability of these compounds to inactivate the AGT protein in HT29 human colon tumor cell extracts and in intact HT29 cells is summarized in Tables 1 and 2. The data represent the dose of compound required to produce 50% inactivation in cell-free extracts upon incubation for 30 min or in cells upon incubation for 4 hr.

Table 1. AGT-Inactivating Activity of 6-Benzyloxypurine, 6(4)-Benzyloxypyrimidine, and 6-Benzyloxy-*s*-triazine Derivatives

	ED_{50} (μM)[a]	
Compound	In HT29 cell-free extract	In HT29 cells
2,4-diamino-6-benzyloxy-5-nitrosopyrimidine **(3d)**	0.06	0.02
2,4-diamino-6-benzyloxy-5-nitropyrimidine **(3e)**	0.06	0.02
8-aza-O^6-benzylguanine **(2)**	0.07	0.06
O^6-benzyl-8-bromoguanine **(1d)**	0.08	0.06
O^6-benzylguanine	0.2	0.05
O^6-benzyl-8-methyl-guanine **(1b)**	0.3	0.1
O^6-benzyl-8-oxoguanine **(1c)**	0.3	0.15
O^6-benzyl-8-trifluoromethylguanine **(1e)**	0.4	0.25
2,4,5-triamino-6-benzyloxypyrimidine **(3c)**	0.4	0.3
2-amino-4-benzyloxy-6-methyl-5-nitropyrimidine **(5b)**	0.4	0.06
2-amino-4-benzyloxy-5-nitropyrimidine **(5a)**	0.4	0.05
8-amino-O^6-benzylguanine **(1a)**	0.7	2
2,4-diamino-6-benzyloxy-5-bromopyrimidine **(3f)**	2	0.8
2,4-diamino-6-benzyloxy-*s*-triazine **(6)**	4	1.0
2,4-diamino-6-benzyloxypyrimidine **(3b)**	15	5
O^6-benzyluric acid **(4b)**	25	45
4-amino-6-benzyloxy-5-nitropyrimidine **(3a)**	28	8
O^6-benzyl-2-fluorohypoxanthine **(4c)**	48	12
O^6-benzylxanthine **(4a)**	60	35
N^2-acetyl-O^6-benzyl-8-oxoguanine **(4d)**	65	11
O^6-benzyl-N^2-methylguanine **(4e)**	160	60
O^6-benzyl-N^2,N^2-dimethylguanine **(4f)**	200	110

[a] The effective dose required to produce 50% inactivation in cell-free extracts upon incubation for 30 min or in cells upon incubation for 4 hr. The values for O^6-benzylguanine are from Moschel et al., J. Med. Chem., 35, 4486-4491 (1992).

Within these series of compounds, O^6-benzyl-N^2-methyl- and O^6-benzyl-N^2,N^2-dimethylguanine (**4e** and **4f**) were the least active agents exhibiting ED_{50} values for inactivation of AGT in HT29 cell extracts of 160 and 200 μM, respectively. For comparison, the ED_{50} value exhibited by O^6-benzylguanine was 0.2 μM (Table 1). The other 2- and/or 8-substituted 6-benzyloxypurines, N^2-acetyl-O^6-benzyl-8-oxoguanine (**4d**), O^6-benzylxanthine (**4a**), O^6-benzyl-2-fluorohypoxanthine (**4c**) and O^6-benzyluric acid (**4b**), together with the substituted pyrimidines 4-amino-6-benzyloxy-5-nitropyrimidine (**3a**) and 2,4-diamino-6-benzyloxypyrimidine (**3b**), comprised a group of increasingly more active AGT inactivating agents exhibiting intermediate ED_{50} values in the range of 65 to 15 μM. 2,4-Diamino-6-benzyloxy-*s*-triazine (**6**) and 2,4-diamino-6-benzyloxy-5-bromopyrimidine (**3f**) were considerably more active than **3b** indicating that electron-withdrawing groups at the 5-position of a 2,4-diamino-6-benzyloxypyrimidine derivative are positive contributors to efficient AGT inactivation. This is further emphasized by the very high activity exhibited by 2,4-diamino-6-benzyloxy-5-nitrosopyrimidine (**3d**) and 2,4-diamino-6-benzyloxy-5-nitropyrimidine (**3e**), which contain strongly electron-withdrawing nitroso and nitro substituents, respectively. These two derivatives are the most active AGT inactivators tested to date. The observation that 2-amino-4-benzyloxy-5-nitropyrimidine (**5a**) is much more active than **3a** indicates that a 2-amino group is critical for high activity for a 6(4)-benzyloxy-5-nitropyrimidine derivative. An additional alkyl group at the 4(6)-position (e.g., as in **5b**) does not enhance activity significantly over that for **5a** although an amino group at the 4(6)-position significantly enhances activity.

Thus, AGT inactivating activity increases substantially over the series **5a**=**5b**<**3d**=**3e**. With these considerations in mind the activity of 2,4,5-triamino-6-benzyloxypyrimidine (**3c**) seems exceptional and the reasons for its relatively high activity are unclear at present. It is also significant that pyrimidines **5a** and **5b** are quite active in cells, which is not totally predicted by their corresponding activity in HT29 cell extracts.

All the O^6-benzylguanine analogs **1a-d** were much more active than the purines in the series **4a-f** and the activity differences among **1a-d** also reflect enhancements due to introduction of electron withdrawing groups. Thus, activity increased in the series 8-amino-O^6-benzylguanine (**1a**) <O^6-benzyl-8-oxoguanine (**1c**) <O^6-benzyl-8-methylguanine (**1b**) <O^6-benzyl-8-bromoguanine (**1d**) <8-aza-O^6-benzylguanine (**2**). Indeed, derivatives **1d** and **2** were essentially as active as pyrimidines **3d** and **3e** in cell-free extracts although **1d** and **2** were somewhat less active in cells than expected from their activity in cell-free extracts.

The compounds listed in Table 2 also had AGT-inactivating activity in cell free extracts and in cells. The activity of **7**, **8a**, and **10a** in cells is significantly higher than their activity in cell-free extracts. Thus the ratio of ED_{50} values in cell-free extracts/intact cells is 1.6, 1.6, 1.1, respectively, for derivatives **1d**, **1e**, and **2** (Table 1). This ratio increases to 7.2, 6.3, and 6.3, respectively, for the corresponding methylated derivatives **10a**, **7**, and **8a**. It is believed that the higher activity of the methylated derivatives in the cells is due to the fact that these compounds do not possess readily dissociable hydrogens in the imidazole portion of the purine ring system and therefore they can readily enter the cells as neutral molecules.

Table 2. AGT-Inactivating Activity of 7,8- and 8,9-Disubstituted *O*[6]-Benzylguanine Derivatives and Related Compounds

	ED_{50} value (μM)[a]	
Compound	In HT29 cell-free extract	In HT29 cells
O[6]-benzyl-8-trifluoromethyl-9-methylguanine (**7**)	2.5	0.4
8-aza-*O*[6]-benzyl-9-methylguanine (**8a**)	0.5	0.08
8-aza-*O*[6]-benzyl-9-(pivaloyloxymethyl)guanine (**8b**)	0.28[b]	0.23
8-aza-*O*[6]-benzyl-7-(pivaloyloxymethyl)guanine (**9**)	0.11[c]	0.16
O[6]-benzyl-8-bromo-9-methylguanine (**10a**)	1.9	0.25
O[6]-benzyl-8-bromo-9-(pivaloyloxymethyl)guanine (**10b**)	0.08[d]	0.05
O[6]-benzyl-7-(pivaloyloxymethyl)guanine (**12**)	9[e]	0.3
O[6]-benzyl-9-(pivaloyloxymethyl)guanine	3.1[f,g]	0.3
O[6]-benzyl-9-methylguanine	2.6[g]	0.4

[a] The dose required to produce 50% inactivation in cell-free extracts upon incubation for 30 min. or in cells upon incubation for 4 hr. [b]ED_{50}=4 with purified human AGT. [c]ED_{50}=2 with purified human AGT. [d]ED_{50} > 100 with purified human AGT. [e]ED_{50} >> 100 with purified human AGT. [f]ED_{50} = 95 with purified human AGT. [g]Data from Chae *et al.*, *J. Med. Chem.*, **37**, 342-347 (1994).

The ability of increasing concentrations of **1a-d**, **2**, and **3c-e** to enhance the killing of human HT29 colon cancer cells, DU-145 prostate cancer cells, and MCF-71 breast cancer cells by BCNU (40 μM) is shown in Tables 3, 4, and 5, respectively. The data reflect the number of cell colonies that result following exposure to AGT inactivator alone or AGT inactivator 2 hr before exposure to BCNU as described in Dolan et al. (*Proc. Natl. Acad. Sci., U.S.A.*, *87*, 5368-5372 (1990)). Data for *O*[6]-benzylguanine are included for comparison. As indicated, at 10 μM

concentrations, all the 8-substituted purines with the exception of **1a** were as effective as O^6-benzylguanine in enhancing the cytotoxicity of BCNU (40 μM); such treatment killed essentially all the tumor cells. Treatment of the cells with the modified 8-substituted O^6-benzylguanine alone or BCNU alone had no significant effect on cell colony number. The comparatively low activity of **1a** in all but the breast cancer cells may reflect its poor transport into other tumor cell types or its rapid metabolic conversion to an ineffective AGT inactivator. Its ineffective enhancement of BCNU cytotoxicity parallels its relatively poor AGT inactivating ability in colon tumor cells (Table 1).

For the pyrimidines tested, 2,4,5-triamino-6-benzyloxypyrimidine (**3c**) was as effective as the 8-substituted O^6-benzylguanine derivatives and O^6-benzylguanine itself in enhancing BCNU toxicity although the nitroso- and nitropyrimidine derivatives (**3d** and **3e**) were similarly effective at a 4-fold lower dose.

Table 3. Killing of HT-29 Colon Cancer Cells by BCNU Combined with AGT Inactivators

Inactivator	Inactivator Concentration (μM)	BCNU (μM)	Colony Formation per 1000 cells
None		None	435±63
None		40	442±34
O^6-benzylguanine	10	None	431±33
	10	40	13±6
	2.5	40	38±15
	1	40	277±25
8-aza-O^6-benzylguanine **(2)**	10	None	537±48
	10	40	2±1
	1	40	423±42
O^6-benzyl-8-bromoguanine **(1d)**	10	None	401±22
	10	40	1±0
	1	40	299±30
O^6-benzyl-8-oxoguanine **(1c)**	10	None	401±22
	10	40	<1
	1	40	221±15
O^6-benzyl-8-methylguanine **(1b)**	10	None	513±76
	10	40	<1
	1	40	230±51
O^6-benzyl-8-aminoguanine **(1a)**	10	None	504±30
	10	40	430±41
	1	40	475±26
2,4,5-triamino-6-benzyloxypyrimidine **(3c)**	10	None	453±59
	10	40	3±1
	1	40	487±32
2,4-diamino-6-benzyloxy-5-nitrosopyrimidine **(3d)**	2.5	None	528±64
	2.5	40	<1
	1	40	19±4
2,4-diamino-6-benzyloxy-5-nitropyrimidine **(3e)**	2.5	None	438±25
	2.5	40	<1
	1	40	45±4

Table 4. Killing of DU-145 Prostate Cancer Cells by BCNU Combined with AGT Inactivators

Inactivator	Inactivator Concentration (μM)	BCNU (μM)	Colony Formation per 1000 cells
None		None	453±81
None		40	394±76
O^6-benzylguanine	10	None	462±68
	10	40	28±5
	1	40	299±18
8-aza-O^6-benzylguanine **(2)**	10	None	452±72
	10	40	28±5
	1	40	248±21
O^6-benzyl-8-bromoguanine **(1d)**	10	None	493±90
	10	40	16±3
	1	40	267±39
O^6-benzyl-8-oxoguanine **(1c)**	10	None	379±34
	10	40	34±3
	1	40	329±43
O^6-benzyl-8-methylguanine **(1b)**	10	None	357±43
	10	40	50±7
	1	40	306±157
O^6-benzyl-8-aminoguanine **(1a)**	10	None	380±36
	10	40	435±70
	1	40	295±45
2,4,5-triamino-6-benzyloxypyrimidine **(3c)**	10	None	429±101
	10	40	57±7
	1	40	378±60
2,4-diamino-6-benzyloxy-5-nitrosopyrimidine **(3d)**	2.5	None	403±35
	2.5	40	7±3
	1	40	25±4
	0.25	40	192±17
2,4-diamino-6-benzyloxy-5-nitropyrimidine **(3e)**	2.5	None	407±80
	2.5	40	9±2
	1	40	59±6
	0.25	40	129±26

Tabl 5. Killing of MCF-71 Breast Cancer Cells by BCNU Combined with AGT Inactivators

Inactivator	Inactivator Concentration (μM)	BCNU (μM)	Colony Formation per 1000 cells
None		None	426±78
None		40	364±60
O^6-benzylguanine	10	None	455±63
	10	40	4±2
	2.5	40	12±6
8-aza-O^6-benzylguanine **(2)**	10	None	483±27
	10	40	2±1
O^6-benzyl-8-bromoguanine **(1d)**	10	None	380±109
	10	40	3±1
	2.5	40	4±3
O^6-benzyl-8-oxoguanine **(1c)**	10	None	522±78
	10	40	4±2
O^6-benzyl-8-methylguanine **(1b)**	10	None	376±76
	10	40	2±1
O^6-benzyl-8-aminoguanine **(1a)**	10	None	432±36
	10	40	95±8
2,4,5-triamino-6-benzyloxypyrimidine **(3c)**	10	None	448±55
	10	40	12±4
2,4-diamino-6-benzyloxy-5-nitrosopyrimidine **(3d)**	2.5	None	447±87
	2.5	40	2±1
2,4-diamino-6-benzyloxy-5-nitropyrimidine **(3e)**	2.5	None	314±49
	2.5	40	2±1

Although the human alkyltransferase is very sensitive to inactivation by O^6-benzylguanine and the various compounds described above, a number of mutants have been generated that are resistant to O^6-benzylguanine (Crone and Pegg, *Cancer Res.*, *53*, 4750-4753 (1993)). This resistance is probably caused by a reduction in the space surrounding the active site of the alkyltransferase, which limits the access to O^6-benzylguanine. These mutants are produced by single base changes in the alkyltransferase DNA-coding sequence causing changes in one or two amino acids in the alkyltransferase (Crone and Pegg, *Cancer Res.*, *53*, 4750-

4753 (1993)). Thus, as indicated in Table 6, changing the proline residue at position 140 to alanine (protein P140A) or the glycine residue at position 156 to an alanine (protein G156A) causes a 20-fold and a 240-fold increase in resistance to O^6-benzylguanine, respectively. The alkyltransferase containing an arginine in place of a proline at residue 138 together with an arginine in place of a proline at residue 140 (protein P138A/P140A) is 88-fold more resistant to inactivation by O^6-benzylguanine. It is possible that such resistant mutants will arise or be selected for in tumors under the selective pressure generated by treatment with O^6-benzylguanine plus an alkylating agent. More potent inhibitors and/or those of a smaller size that are better able to fit into the space of the active site of the mutant alkyltransferase can be used to advantage to overcome this resistance.

Table 6. Inhibition of Mutant Alkyltransferase Proteins by O^6-Benzylguanine or 2,4-Diamino-6-benzyloxy-5-nitrosopyrimidine

Protein	ED_{50} value (μM)[a]	
	O^6-benzylguanine	2,4-diamino-6-benzyloxy-5-nitroso-pyrimidine
Control	0.25	0.05
P140A	5	0.1
P138A/P140A	22	0.3
G156A	60	1

[a] The concentration needed to inactivate 50% of the activity in 30 minutes.

As shown in Table 6, 2,4-diamino-6-benzyloxy-5-nitrosopyrimidine (**3d**) was 50 to 60 times better at inactivating the mutant alkyltransferases than O^6-benzylguanine. Doses of 2,4-diamino-6-benzyloxy-5-nitrosopyrimidine leading to intracellular concentrations greater than 5 μM will therefore be effective at

inactivating such resistant alkyltransferases. Concentrations greater than 200 μM of O^6-benzylguanine would be needed to get such inactivation, and these are much more than can be achieved with this compound in current formulations. However, 8-substituted O^6-benzylguanine derivatives that are significantly more potent than O^6-benzylguanine may be useful in inactivating mutant alkyltransferases provided their required intracellular concentrations can be achieved. These data for mutant alkyltransferase inactivation and the data presented earlier indicate that pyrimidine derivatives bearing electron-withdrawing groups at the 5-position as well as substituted O^6-benzylguanine derivatives bearing electron-withdrawing groups at the 8-position are superior to O^6-benzylguanine for use as adjuvants in chemotherapy with agents whose mechanism of action, like that of BCNU, involves modification of the O^6-position of DNA guanine residues.

Other 8-substituted O^6-benzylguanine derivatives bearing electron-withdrawing 8-substituents (e.g., NO_2) are readily available. For example, O^6-benzyl-8-nitroguanine could be prepared by treatment of 8-nitroguanine (Jones and Robins, *J. Am. Chem. Soc., 82*, 3773-3779 (1960)) with phosphorus oxychloride to produce 2-amino-6-chloro-8-nitropurine which when treated with sodium benzyloxide in benzyl alcohol would produce the desired O^6-benzyl-8-nitroguanine.

Additional 2,4-diamino-6-benzyloxypyrimidine derivatives bearing electron-withdrawing groups other than halogen or nitro groups (e.g., formyl or cyano groups) could also be readily prepared. 2,4-Diamino-5-formyl-6-hydroxypyrimidine, a known compound (Delia and Otteman, *Heterocycles, 20*, 1805-1809 (1983)), can be treated with phosphorus oxychloride to produce a 2,4-diamino-6-chloro-5-formylpyrimidine intermediate, which on treatment with sodium benzyloxide in benzyl alcohol produces 2,4-diamino-6-benzyloxy-5-formylpyrimidine. Treatment of the formyl

pyrimidine with hydroxylamine affords 2,4-diamino-6-benzyloxy-5-cyanopyrimidine. The preparation of a large number of 5-substituted 6(4)-benzyloxypyrimidines or 8-substituted O^6-benzylguanine derivatives is possible for those skilled in the art of synthesis of heterocyclic aromatic compounds (D.J. Brown, "The Pyrimidines," in *The Chemistry of Heterocyclic Compounds*, Vol. 16, A. Weissberger, Ed., Wiley Interscience, New York, 1962; D.J. Brown, "The Pyrimidines," Supplement I, in *The Chemistry of Heterocyclic Compounds*, Vol. 16, A. Weissberger and E.C. Taylor, Eds., Wiley Interscience, New York, 1970; J.H. Lister, "Fused Pyrimidines Part II Purines," in *The Chemistry of Heterocyclic Compounds*, Vol. 24 Part II, A. Weissberger and E.C. Taylor, Eds., Wiley Interscience, New York, 1971).

Because many 9-substituted O^6-benzylguanine derivatives exhibit excellent AGT inactivation properties (Moschel et al., *J. Med. Chem.*, *35*, 4486-4491 (1992); Chae et al., *J. Med. Chem.*, *37*, 342-347 (1994)), 8,9-disubstituted analogs are expected to be similarly active. These can be readily prepared by reacting the anion of 8-substituted O^6-benzylguanines (e.g., **1a-e**) or the anion of 8-aza-O^6-benzylguanine (**2**) with any of the range of compounds already described (Moschel et al., *J. Med. Chem.*, *35*, 4486-4491 (1992); Chae et al., *J. Med. Chem.*, *37*, 342-347 (1994)) to produce a mixture of isomeric 7,8- and 8,9-disubstituted O^6-benzylguanine derivatives. The desired 8,9-disubstituted derivative can be isolated and purified by silica gel column chromatography as already described (Moschel et al., *J. Med. Chem.*, *35*, 4486-4491 (1992); Chae et al., *J. Med. Chem.*, *37*, 342-347 (1994)). Compound **7** was prepared by treating the anion of compound **1e** with methyl iodide in *N,N*-dimethylformamide. Compounds **8-12** were prepared using similar procedures.

The O^6-substituted compounds of the present invention can be administered in any suitable manner to a mammal for the purpose of enhancing the chemotherapeutic treatment of

a particular cancer. Although more than one route can be used to administer a particular compound, a particular route can provide a more immediate and more effective reaction than another route. Accordingly, the described methods provided herein are merely exemplary and are in no way limiting.

Generally, the O^6-substituted compounds of the present invention as described above will be administered in a pharmaceutical composition to an individual afflicted with a cancer. Those undergoing or about to undergo chemotherapy can be treated with the O^6-substituted compounds separately or in conjunction with other treatments, as appropriate. In therapeutic applications, compositions are administered to a patient in an amount sufficient to elicit an effective depression of AGT activity thereby potentiating the cytotoxicity of the aforedescribed chemotherapeutic treatment. An amount adequate to accomplish this is defined as a "therapeutically effective dose," which is also an "AGT inactivating effective amount." Amounts effective for a therapeutic or prophylactic use will depend on, e.g., the stage and severity of the disease being treated, the age, weight, and general state of health of the patient, and the judgment of the prescribing physician. The size of the dose will also be determined by the O^6-substituted compound selected, method of administration, timing and frequency of administration as well as the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular O^6-substituted compound and the desired physiological effect. It will be appreciated by one of skill in the art that various disease states may require prolonged treatment involving multiple administrations, perhaps using a series of different AGT inactivators and/or chemotherapeutic agents in each or various rounds of administration.

Suitable chemotherapeutic agents usefully administered in coordination with the O^6-substituted compounds of the

present invention include alkylating agents, such as chloroethylating and methylating agents. Such agents may be administered using conventional techniques such as those described in Wasserman et al., Cancer, 36, pp. 1258-1268 (1975), and Physicians' Desk Reference, 48th ed., Edward R. Barnhart publisher (1994). For example, 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine or BCNU, Bristol-Myers, Evansville, IN) may be administered intravenously at a dosage of from about 150 to 200 mg/m^2 every six weeks. Another alkylating agent, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (lomustine or CCNU, Bristol-Myers), may be administered orally at a dosage of about 130 mg/m^2 every six weeks. Other alkylating agents may be administered in appropriate dosages via appropriate routes of administration known to skilled medical practitioners.

Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, treatment is initiated with smaller dosages that are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. The present inventive method typically will involve the administration of about 0.1 μg to about 50 mg of one or more of the compounds described above per kg body weight of the individual. For a 70 kg patient, dosages of from about 10 μg to about 200 mg of O^6-substituted compound would be more commonly used, possibly followed by further lesser dosages from about 1 μg to about 1 mg of O^6-substituted compound over weeks to months, depending on a patient's physiological response, as determined by measuring cancer-specific antigens or other measurable parameters related to the tumor load of a patient.

It must be kept in mind that the compounds and compositions of the present invention generally are employed in serious disease states, that is, life-threatening or potentially life-threatening situations. In

such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the O^6-substituted compounds, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these O^6-substituted compounds.

Single or multiple administrations of the compounds can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of AGT-inactivating compounds of the invention sufficient to effectively enhance the cytotoxic impact of the chemotherapy.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral or local administration and generally comprise a pharmaceutically acceptable carrier and an amount of the active ingredient sufficient to reduce, and preferably prevent, the activity of the AGT protein. The carrier may be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the compound, and by the route of administration.

Examples of pharmaceutically acceptable acid addition salts for use in the present inventive pharmaceutical composition include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, *p*-toluenesulphonic acids, and arylsulphonic, for example.

The pharmaceutically acceptable excipients described herein, for example, vehicles, adjuvants, carriers or diluents, are well-known to those who are skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one that is chemically inert to the active compounds and one that has no detrimental side effects or toxicity under

the conditions of use. Such pharmaceutically acceptable excipients preferably include saline (e.g., 0.9% saline), Cremophor EL (which is a derivative of castor oil and ethylene oxide available from Sigma Chemical Co., St. Louis, MO) (e.g., 5% Cremophor EL/5% ethanol/90% saline, 10% Cremophor EL/90% saline, or 50% Cremophor EL/50% ethanol), propylene glycol (e.g., 40% propylene glycol/10% ethanol/50% water), polyethylene glycol (e.g., 40% PEG 400/60% saline), and alcohol (e.g., 40% t-butanol/60% water). The most preferred pharmaceutical excipient for use in conjunction with the present invention is polyethylene glycol, such as PEG 400, and particularly a composition comprising 40% PEG 400 and 60% water or saline.

The choice of excipient will be determined in part by the particular O^6-substituted compound chosen, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present invention.

The following formulations for oral, aerosol, parenteral, subcutaneous, intravenous, intraarterial, intramuscular, interperitoneal, rectal, and vaginal administration are merely exemplary and are in no way limiting.

The pharmaceutical compositions can be administered parenterally, e.g., intravenously, intraarterially, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration that comprise a solution of the O^6-substituted compound dissolved or suspended in an acceptable carrier suitable for parenteral administration, including aqueous and non-aqueous, isotonic sterile injection solutions.

Overall, the requirements for effective pharmaceutical carriers for parenteral compositions are well known to those of ordinary skill in the art. See Pharmaceutics and Pharmacy Practice, J.B. Lippincott Company, Philadelphia,

PA, Banker and Chalmers, eds., pages 238-250 (1982), and ASHP Handbook on Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986). Such solutions can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compound may be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

Oils useful in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils useful in such formulations include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic

detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

The parenteral formulations typically will contain from about 0.5% to about 25% by weight of the active ingredient in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

Topical formulations, including those that are useful for transdermal drug release, are well-known to those of skill in the art and are suitable in the context of the present invention for application to skin.

Formulations suitable for oral administration require extra considerations considering the peptidyl and/or carbohydrate nature of some of the O^6-substituted compounds of the present invention and the likely breakdown thereof

if such compounds are administered orally without protecting them from the digestive secretions of the gastrointestinal tract. Such a formulation can consist of (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent, or emulsifying agent. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible excipients. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such excipients as are known in the art.

The O^6-substituted compounds of the present invention, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. The compounds are preferably supplied in

finely divided form along with a surfactant and propellant. Typical percentages of active compound are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such surfactants are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included as desired, e.g., lecithin for intranasal delivery. These aerosol formulations can be placed into acceptable pressurized propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations may be used to spray mucosa.

Additionally, the compounds and polymers useful in the present inventive methods may be made into suppositories by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

The concentration of the O^6-substituted compounds of the present invention in the pharmaceutical formulations can vary widely, i.e., from less than about 1%, usually at or at least about 10%, to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 100 mg of the O^6-substituted compound. Actual methods for preparing parenterally administrable compounds will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science (17th ed., Mack Publishing Company, Easton, PA, 1985).

It will be appreciated by one of ordinary skill in the art that, in addition to the aforedescribed pharmaceutical compositions, the O^6-substituted compounds of the present inventive method may be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes. Liposomes serve to target the compounds to a particular tissue, such as lymphoid tissue or cancerous hepatic cells. Liposomes can also be used to increase the half-life of the O^6-substituted compound. Liposomes useful in the present invention include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the O^6-substituted compound to be delivered is incorporated as part of a liposome, alone or in conjunction with a suitable chemotherapeutic agent. Thus, liposomes filled with a desired O^6-substituted compound of the invention can be directed to the site of a specific tissue type, hepatic cells, for example, where the liposomes then deliver the selected chemotherapeutic-enhancement compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, for example, liposome size and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, for example, Szoka et al., *Ann. Rev. Biophys. Bioeng.*, *9*, 467 (1980), and U.S. Patents 4,235,871, 4,501,728, 4,837,028, and 5,019,369. For targeting to the

cells of a particular tissue type, a ligand to be incorporated into the liposome can include, for example, antibodies or fragments thereof specific for cell surface determinants of the targeted tissue type. A liposome suspension containing an O^6-substituted compound may be administered intravenously, locally, topically, etc. in a dose that varies according to the mode of administration, the O^6-substituted compound being delivered, the stage of disease being treated, etc.

While the efficacy of the O^6-substituted compounds of the present invention has been demonstrated with respect to particular types of cancerous cells, e.g., colon, prostate, and breast cancer cells, the present invention has applicability to the treatment of any type of cancer capable of being treated with an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine. Such cancers include, for example, colon tumors, prostrate tumors, brain tumors, lymphomas, leukemias, breast tumors, ovarian tumors, lung tumors, Wilms' tumor, rhabdomyosarcoma, multiplemyeloma, stomach tumors, soft-tissue sarcomas, Hodgkin's disease, and non-Hodgkin's lymphomas.

Similarly, in view of the mode of action of the O^6-substituted compounds of the present invention, such compounds can be used in conjunction with any type of antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine. Such antineoplastic alkylating agents include, for example, chloroethylating agents (e.g. chloroethylnitrosoureas and chloroethyltriazines) and monofunctional alkylating agents such as Streptozotocin, Procarbazine, Dacarbazine, and Temozolomide.

Among the chloroethylating agents, the most frequently used chemotherapeutic drugs are 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, lomustine), 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU, carmustine), 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU,

semustine), and 1-(2-chloroethyl)-3-(4-amino-2-methyl-5-pyrimidinyl)methyl-1-nitrosourea (ACNU). These agents have been used clinically against tumors of the central nervous system, multiple myeloma, melanoma, lymphoma, gastrointestinal tumors, and other solid tumors (Colvin and Chabner, Alkylating Agents. *In*: Cancer Chemotherapy: Principles and Practice, Chabner and Collins, eds., Lippincott, Philadelphia, pp. 276-313 (1990); McCormick and McElhinney, *Eur. J. Cancer, 26*, 207-221 (1990)). Chloroethylating agents currently under development with fewer side effects are 1-(2-chloroethyl)-3-(2-hydroxyethyl)-1-nitrosourea (HECNU), 2-chloroethyl-methylsulfonylmethanesulfonate (Clomesone), and 1-[N-(2-chloroethyl)-N-nitrosoureido]ethylphosphonic acid diethyl ester (Fotemustine) (Colvin and Chabner, Alkylating Agents. *In*: Cancer Chemotherapy: Principles and Practice, Chabner and Collins, eds., Lippincott, Philadelphia, pp. 276-313 (1990); McCormick and McElhinney, *Eur. J. Cancer, 26*, 207-221 (1990)). Methylating chemotherapeutic agents include Streptozotocin (2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose), Procarbazine (N-(1-methylethyl)-4-[(2-methylhydrazino)methyl]benzamide), Dacarbazine or DTIC (5-(3,3-dimethyl-1-triazenyl)-1H-imidazole-4-carboxamide),and Temozolomide (8-carbamoyl-3-methylimidazo[5,1-d]-1,2,3,5-tetrazine-4-(3H)-one). Temozolomide is active against malignant melanomas, brain tumors, and mycosis fungoides. Streptozotocin is effective against pancreatic tumors. Procarbazine is used to treat Hodgkin's disease and brain tumors, and DTIC is used in treatment of melanoma and lymphomas (Colvin and Cabner, Alkylating Agents. *In*: Cancer Chemotherapy: Principles and Practice, Chabner and Collins, eds., Lippincott, Philadelphia, pp. 276-313 (1990); Longo, *Semin. Concol., 17*, 716-735 (1990)).

The examples set forth below describe the syntheses of the aforedescribed compounds. As regards the methods and materials set forth in these examples, ^{1}H-NMR spectra were recorded on a Varian VXR 500S spectrometer equipped with

Sun 2/110 data stations or a Varian XL 200 instrument interfaced to an Advanced data system. Samples were dissolved in DMSO-d_6 with Me_4Si as an internal standard. EI mass spectra were obtained on a reversed geometry VG Micromass ZAB-2F spectrometer interfaced to a VG 2035 data system. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Most of the reagents and solvents were from Aldrich Chemical Co., Inc., Milwaukee, WI. 8-Aza-O^6-benzylguanine (**2**) (Shealy et al., *J. Org. Chem.*, *27*, 4518-4523 (1962)), 2,4-diamino-6-benzyloxypyrimidine (**3b**) (Pfleiderer and Lohrmann, *Chem. Ber.*, *94*, 12-18 (1961)), 2,4,5-triamino-6-benzyloxypyrimidine (**3c**) (Pfleiderer and Lohrmann, *Chem. Ber.*, *94*, 12-18 (1961)), 2,4-diamino-6-benzyloxy-5-nitrosopyrimidine (**3d**) (Pfleiderer and Lohrman, *Chem. Ber.*, *94*, 12-18 (1961)), 2,4-diamino-6-benzyloxy-5-nitropyrimidine (**3e**) (Kosary et al., *Acta Pharm. Hung.*, *49*, 241-247 (1989)), 2,4-diamino-6-benzyloxy-5-bromopyrimidine (**3f**) (Kosary et al., *Acta Pharm. Hung.*, *49*, 241-247 (1989)), 4-amino-6-benzyloxy-5-nitropyrimidine (**3a**) and O^6-benzyl-2-fluorohypoxanthine (**4c**) (Robins and Robins, *J. Org. Chem.*, *34*, 2160-2163 (1969)) were prepared previously. Alternative synthetic methods are provided below for some of these compounds together with spectroscopic data not provided previously. AGT inactivation studies were carried out as described in Moschel et al., *J. Med. Chem.*, *35*, 4486-4491 (1992). Cell killing experiments involving various AGT inactivators in combination with BCNU were carried out as in Dolan, et al. (*Proc. Natl. Acad. Sci. U.S.A.*, *87*, 5368-5372 (1990)). Cells were treated for 2 h with AGT inactivator prior to exposure to BCNU.

<u>Example 1</u>: **2,8-Diamino-6-chloropurine**

A suspension of 8-aminoguanine (Fischer, *Z. Physiol. Chem.*, *60*, 69 (1909); Beaman et al., in Zorbach and Tipson, *Synthetic Procedures in Nucleic Acid Chemistry*, Vol. 1, pp 41 - 43, John Wiley & Sons, New York, 1968) (3.0 g, 18.1

mmol) in phosphorus oxychloride (90 mL) and *N,N*-diethylaniline (3 mL) was refluxed for 30 min and the excess phosphorus oxychloride was evaporated under reduced pressure. Ice (20 g) was added slowly to the resulting solution and the pH was adjusted to 6 with a concentrated aqueous sodium hydroxide solution. A yellow solid formed and was collected by filtration, washed with water, and dried to give a green solid. Crystallization from water with charcoal treatment produced 2,8-diamino-6-chloropurine as a white solid: yield, 2.11 g (63%); mp >275 °C dec.; ^{1}H NMR δ 6.09 (s, 2 H, NH_2, exchange with D_2O), 6.71 (s, 2 H, NH_2, exchange with D_2O); MS (EI) calcd. *m/z* for $C_5H_5N_6{}^{35}Cl$ 184.0264, found 184.0266; calcd. *m/z* $C_5H_5N_6{}^{37}Cl$ 186.0235, found 186.0237.

Example 2: **8-Amino-O^6-benzylguanine (1a)**

2,8-Diamino-6-chloropurine (0.9 g, 4.9 mmol) was added to the solution of sodium (0.22 g, 10 mmol) in benzyl alcohol (9.0 mL). The solution was heated in a 130 °C oil bath for 5 h, and was poured into water (100 mL) with constant stirring for 10 min. Undissolved solid was removed by filtration and the filtrate was neutralized with glacial acetic acid. The solution was mixed with methanol (100 mL), and half of the aqueous methanol solution was loaded on a 3 x 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm and fractions (10 mL) were collected. The remainder of the reaction mixture in $MeOH/H_2O$ was chromatographed separately under identical conditions. The desired product eluted in fractions 100 - 130. Evaporation of solvent from the pooled fractions 100-130 from both chromatographic runs afforded analytically pure **1a**: yield, 0.26 g (21%); mp 269 - 271 °C dec.; UV (pH 1) λ_{max} 241 nm (ϵ = 0.699 x 10^4), 300 (1.109 x 10^4); (pH 6.9) 250 (sh) (0.447 x 10^4), 292 (1.027 x 10^4); (pH 13) 255 (sh) (0.355 x 10^4), 295 (0.932 x 10^4); ^{1}H NMR δ 5.41 (s, 2 H, $ArCH_2$), 5.70 (s, 2 H, NH_2, exchange with D_2O), 6.18 (s,

2 H, NH_2, exchange with D_2O), 7.25-7.55 (m, 5 H, ArH), 11.1 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. *m/z* for $C_{12}H_{12}N_6O$ 256.1072, found 256.1059; Anal. ($C_{12}H_{12}N_6O$) C, H, N.

Example 3: **2-Amino-6-chloro-8-methylpurine**

A suspension of 8-methylguanine (Daves et al., *J. Am. Chem. Soc.*, *82*, 2633 - 2640 (1960)) (1.0 g, 6.1 mmol) in phosphorous oxychloride (30 mL) and *N,N*-diethylaniline (1 mL) was refluxed for 3 h. The excess phosphorous oxychloride was evaporated under reduced pressure. The resulting brown oil was dissolved in ice-water and was neutralized with a concentrated aqueous NaOH solution. After evaporation of the solvent, the solid residue was suspended in 70 mL of H_2O. Undissolved solid was filtered off, and the filtrate was loaded on a 3 x 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm and fractions (10 mL) were collected. Evaporation of pooled fractions 50-60 produced 2-amino-6-chloro-8-methylpurine as a crude solid. Crystallization from ethanol/water with charcoal treatment afforded 2-amino-6-chloro-8-methylpurine as a white solid: yield, 0.57 g (51%); mp > 265 °C dec.; ^1H NMR δ 2.39 (s, 3 H, CH_3), 6.62 (s, 2 H, NH_2, exchange with D_2O), 12.56 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. *m/z* for $C_6H_6N_5{}^{35}Cl$ 183.0312, found 183.0309; calcd. *m/z* for $C_6H_6N_5{}^{37}Cl$ 185.0283, found 185.0286.

Example 4: **O^6-Benzyl-8-methylguanine (1b)**

Sodium (0.1 g, 4.4 mmol) was stirred in 4.1 mL of benzyl alcohol until all sodium had reacted. 2-Amino-6-chloro-8-methylpurine (0.41 g, 2.2 mmol) was added, and the reaction mixture was heated in a 130 °C oil bath for 5 h. After cooling to room temperature 40 mL of ether was added to remove excess benzyl alcohol. The sticky precipitate that formed was collected by filtration and was dissolved in water (50 mL). The pH of the yellow solution was

adjusted to 5 - 6 with glacial acetic acid. The solution was mixed with methanol (50 mL) and was loaded on a 3 x 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm and fractions (10 mL) were collected. Evaporation of pooled fractions 78-93 afforded analytically pure **1b**: yield, 0.25 g (44%); mp 214 - 216 °C; UV (pH 1) λ_{max} 238 nm (sh) (ϵ = 0.648 x 10^4), 290 (1.136 x 10^4); (pH 6.9) 242 (0.758 x 10^4), 284 (0.897 x 10^4); (pH 13) 240 (sh) (0.495 x 10^4), 286 (0.932 x 10^4); ^{1}H NMR δ 2.33 (s, 3 H, CH_3), 5.46 (s, 2 H, $ArCH_2$), 6.17 (s, 2 H, NH_2, exchange with D_2O), 7.34-7.51 (m, 5 H, ArH), 12.18 (br s, 1 H, NH, exchanges with D_2O),; MS (EI) calcd. *m/z* for $C_{13}H_{13}N_5O$ 255.1120, found 255.1125; Anal. ($C_{13}H_{13}N_5O$.1/4 H_2O) C, H, N.

<u>Example 5</u>: ***O*^{6}-Benzyl-8-oxoguanine (1c)**

2,4,5-Triamino-6-benzyloxypyrimidine (Pfleiderer et al., *Chem. Ber.*, *94*, 12 - 18 (1961)) (1.85 g, 8 mmol) and 1,1'-carbonyldiimidazole (1.30 g, 8 mmol) were dissolved in anhydrous *N,N*-dimethylformamide (5 mL) under argon. The solution was stirred at room temperature overnight and was mixed with water (200 mL) to precipitate a white solid. The solid was collected by filtration, and dissolved in 250 mL of aqueous 2 N NaOH solution. Undissolved material was removed by filtration, and the filtrate was neutralized with glacial acetic acid to precipitate a white solid. The solid was collected by filtration, was washed with water, and was recrystallized from 50% aqueous ethanol to afford analytically pure **1c**: yield, 1.63 g (79 %); mp 256 - 257 °C dec.; UV (pH 1) λ_{max} 243 nm (ϵ = 0.717 x 10^4), 306 (1.499 x 10^4); (pH 6.9) 243 (0.915 x 10^4), 290 (1.108 x 10^4); (pH 13) 249 (sh) (0.443 x 10^4), 293 (1.368 x 10^4); ^{1}H NMR δ 5.41 (s, 2 H, $ArCH_2$), 6.13 (s, 2 H, NH_2, exchange with D_2O), 7.33 - 7.51 (m, 5 H, ArH), 10.46 (s, 1 H, exchanges with D_2O), 11.04 (s, 1 H, exchanges with D_2O); MS (EI) Calcd. *m/z* for $C_{12}H_{11}N_5O_2$: 257.0912. Found: 257.0914. Anal. ($C_{12}H_{11}N_5O_2$. 1/2 H_2O) C, N, H.

Example 6: **O^6-Benzyl-8-bromoguanine (1d)**

Bromine (0.26 mL, 5.1 mmol) was added slowly to the solution of O^6-benzylguanine (1.205 g, 5.0 mmol) in anhydrous DMF (10 mL) under argon. The resulting deep green solution was stirred at room temperature overnight. The solution was mixed with water (70 mL) to precipitate crude product. This product was collected by filtration and was dissolved in 50% aqueous methanol (100 mL). The solution was loaded on a 3 x 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm and fractions (10 mL) were collected. The desired product eluted in fractions 110 - 190. Evaporation of solvent from the pooled fractions 110 - 190 afforded **1d** as a pale yellow solid. Crystallization from ethanol/water (1:1) produced analytically pure **1d**: yield, 0.166 g (10%); mp 135 - 137 °C dec.; UV (pH 1) λ_{max} 236 nm (sh) (ϵ = 0.517 x 10^4), 294 (1.429 x 10^4); (pH 6.9) 244 (0.666 x 10^4), 287 (1.043 x 10^4); (pH 13) 245 (sh) (0.544 x 10^4), 289 (1.030 x 10^4); ^1H NMR δ 5.45 (s, 2 H, $ArCH_2$), 6.35 (s, 2 H, NH_2, exchange with D_2O), 7.34 - 7.52 (m, 5 H, ArH), 13.08 (b s, 1 H, NH, exchanges with D_2O) ; MS (EI) calcd. *m/z* for $C_{12}H_{10}N_5O^{79}Br$ 319.0068, found 319.0069; calcd. *m/z* for $C_{12}H_{10}N_5O^{81}Br$ 321.0048, found 321.0048; Anal. ($C_{12}H_{10}N_5OBr3/2\ H_2O$) C, H, N, Br.

Example 7: **8-Aza-O^6-benzylguanine (2)**

Glacial acetic acid (1 mL) was added into the mixture of 2,4,5-triamino-6-benzyloxypyrimidine (0.231 g, 1.0 mmol) and sodium nitrite (0.069 g, 1.0 mmol) in acetone (5 mL). The resulting mixture was stirred at room temperature for 2 h. The solution was poured in water (100 mL) with stirring to precipitate a crude solid. The solid was collected by filtration and air dried. Crystallization from ethanol/water (1:1) with charcoal treatment produced **2** as a white solid: yield, 105 mg (43%); mp 191 - 192 °C (192 - 193 °C; Shealy et. al., *J. Org. Chem.*, *27*, 4518 -

4523 (1962)); ^{1}H NMR δ 5.56 (s, 2 H, $ArCH_2$), 7.00 (s, 2 H, NH_2, exchange with D_2O), 7.41 - 7.58 (m, 5 H, ArH) ; MS (EI) calcd. *m/z* for $C_{11}H_{10}N_6O$ 242.0916, found 242.0924.

Example 8: **4-Amino-6-benzyloxy-5-nitropyrimidine (3a)**

4-Amino-6-chloro-5-nitropyrimidine (Boon et al., *J. Chem. Soc.*, 96-102 (1951)) (1.5 g, 8.6 mmol) was added to a solution of sodium (0.23 g, 9.9 mmol) in benzyl alcohol (14 mL). The solution was heated in a 130 °C oil bath for 3.5 h, and was poured into benzene (50 mL). A yellow solid was collected by filtration and washed with benzene. Crystallization from benzene/ether afforded an analytically pure sample of **3a**: yield, 0.71 g (34%); mp 149 - 150 °C; UV (pH 1) λ_{max} 284 nm (ϵ = 0.368 x 10^4), 333 (0.488 x 10^4); (pH 6.9) 284 (0.329 x 10^4), 336 (0.470 x 10^4); (pH 13) 290 (0.344 x 10^4), 333 (0.494 x 10^4); ^{1}H NMR δ 5.50 (s, 2 H, $ArCH_2$), 7.33 - 7.49 (m, 5 H, ArH), 8.12 - 8.24 (br d, 2 H, NH_a and NH_b, exchange with D_2O), 8.24 (s, 1 H, H-2); MS (EI) calcd. *m/z* for $C_{11}H_{10}N_4O_3$ 246.0752, found 246.0751; Anal. ($C_{11}H_{10}N_4O_3$) C, H, N.

Example 9: **2,4-Diamino-6-benzyloxy-5-nitropyrimidine (3e)**

2,4-Diamino-6-chloro-5-nitropyrimidine (O'Brien et. al., *J. Med. Chem.*, *9*, 573 - 575 (1966)) (1.0 g, 5.28 mmol) was added to a solution of sodium (0.14 g, 6.08 mmol) in benzyl alcohol (9 mL). The solution was heated in a 160 °C oil bath for 3.5 h and the solvent was evaporated under reduced pressure to provide a yellow solid. This solid was washed with water, and air dried. Crystallization from benzene/ether gave a pale yellow filamentous solid: yield, 0.69 g (50 %); mp 194 - 195 °C (171 °C; Kosary et. al., *Acta. Pharm. Hung.*, 49, 241 - 247 (1989)); UV (pH 1) λ_{max} 236 nm (sh) (ϵ = 1.452 x 10^4), 264 (0.522 x 10^4), 321 (1.294 x 10^4); (pH 6.9) 242 (sh) (0.965 x 10^4), 337 (1.493 x 10^4); (pH 13) 242 (sh) (0.952 x 10^4), 338 (1.479 x 10^4); ^{1}H NMR δ 5.43 (s, 2 H, $ArCH_2$), 7.26 (br s, 2 H, NH_2, exchange with D_2O), 7.33 - 7.51 (m, 5 H, ArH), 7.93 (br s, 2 H, NH_2,

exchange with D_2O); MS (EI) calcd. m/z for $C_{11}H_{11}N_5O_3$ 261.0861, found 261.0866; Anal. ($C_{11}H_{11}N_5O_3$).

Example 10: **O^6-Benzylxanthine (4a)**

A suspension of O^6-benzylguanine (0.83 g, 3.4 mmol) in acetone (15 mL) was poured into a solution of sodium nitrite (5 g) in 15 mL of H_2O. Acetic acid (8 mL) was added to the suspension with stirring. Minimum amounts of acetone were added as necessary to dissolve any suspended solid. The resulting pale yellow-green solution was stirred for 3 h. A pale green precipitate that formed was collected by filtration and washed with water (200 mL). Recrystallization of the air-dried solid from ethanol/water (1:1) afforded analytically pure **4a**: yield, 0.43 g (52%); mp 145 - 147 °C dec.; UV (pH 1) λ_{max} 270 nm (ϵ = 0.749 x 10^4); (pH 6.9) 286 (1.143 x 10^4); (pH 13) 290 (0.914 x 10^4); ^1H NMR δ 5.49 (s, 2 H, $ArCH_2$), 7.36-7.54 (m, 5 H, ArH), 8.02 (s, 1 H, H-8), 11.8 (br s, 1 H, NH, exchanges with D_2O), 13.2 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. *m/z* for $C_{12}H_{10}N_4O_2$ 242.0803, found 242.0828; Anal. ($C_{12}H_{10}N_4O_2$. H_2O) C, H, N.

Example 11: **O^6-Benzyluric acid (4b)**

Sodium nitrite (1.5 g, 43 mmol) dissolved in water (5 mL) was added to a suspension of O^6-benzyl-8-oxoguanine (**1c**) (0.257 g, 1.0 mmol) in acetone (5 mL). Glacial acetic acid (3 mL) was added to the suspension with stirring. After stirring for 3 h at room temperature a bright yellow precipitate formed. The suspension was mixed with water (150 mL) and undissolved solid was filtered off. Saturated aqueous sodium carbonate solution was added to the filtrate to adjust the pH to approximately 5. A yellow precipitate (130 mg) was collected and washed with water. This solid was crystallized from 50% aqueous ethanol to give an analytically pure sample of **4b**: yield, 75 mg (29 %); mp >230 °C; UV (pH 1) λ_{max} 236 nm (sh) (ϵ = 0.972 x 10^4), 299 (1.427 x 10^4); (pH 6.9) 240 (sh) (0.821 x 10^4), 304 (2.134

x 10^4); (pH 13) 245 (sh) (0.846 x 10^4), 297 (1.861 x 10^4); ^{1}H NMR δ 5.43 (s, 2 H, $ArCH_2$), 7.35-7.51 (m, 5 H, ArH), 10.76 (s, 1 H, NH, exchanges with D_2O), 11.23 (s, 1 H, NH, exchanges with D_2O), 11.39 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. m/z for $C_{12}H_{10}N_4O_3$ 258.0752, found 258.0753; Anal. ($C_{12}H_{10}N_4O_3$.5/2 H_2O) C, H, N.

Example 12: **Diacetyl-O^6-benzyl-8-oxoguanine**

Acetic anhydride (2 mL) was added to the suspension of O^6-benzyl-8-oxoguanine (**1c**) (0.257 g, 1.0 mmol) in dry toluene (10 mL). The suspension was vigorously refluxed for 24 hr, and was cooled to room temperature. After storing at 4 °C for 4 hr, the resulting precipitate was collected by filtration, washed with benzene and air dried to give an analytically pure sample of a diacetylated product: yield, 0.287 g (84%); mp 272 - 274 °C dec.; UV (100% MeOH) λ_{max} 275 nm (ϵ = 1.313 x 10^4); (pH 1) 275 (1.143 x 10^4); (pH 6.9) 238 (0.995 x 10^4), 276 (1.115 x 10^4); (pH 13) 285 (2.138 x 10^4); ^{1}H NMR δ 2.18 (s, 3 H, CH_3), 2.57 (s, 3 H, CH_3), 5.51 (s, 2 H, $ArCH_2$), 7.30 - 7.57 (m, 5 H, ArH), 10.41 (s, 1 H, exchanges with D_2O), 12.30 (s, 1 H, exchanges with D_2O); MS (EI) Calcd. m/z for $C_{16}H_{15}N_5O_4$: 341.1123. Found: 341.1130. Anal. ($C_{16}H_{15}N_5O_4$) C, N, H.

Example 13: **N^2-Acetyl-O^6-benzyl-8-oxoguanine (4d)**

Diacetyl-O^6-benzyl-8-oxoguanine (85 mg, 0.25 mmol) was dissolved in methanol (10 mL) and ammonium hydroxide (28%, 5 mL) and was allowed stand for 1 hr. The clear solution became cloudy and a precipitate formed on standing. The precipitate was collected by filtration, washed with water, and dried to give an analytically pure sample of **4d**: yield, 48 mg (65%); mp 335 - 337 °C dec.; UV (pH 1) λ_{max} 276 nm (ϵ = 1.723 x 10^4), 303 (sh) (0.679 x 10^4); (pH 6.9) 276 (1.379 x 10^4); (pH 13) 284 (1.683 x 10^4); ^{1}H NMR δ 2.15 (s, 3 H, CH_3), 5.49 (s, 2 H, $ArCH_2$), 7.30 - 7.55 (m, 5 H, ArH), 10.21 (s, 1 H, exchanges with D_2O), 10.99 (s, 1 H, exchanges with D_2O), 11.60 (s, 1 H, exchanges with D_2O; MS (EI) Calcd.

m/z for $C_{14}H_{13}N_5O_3$: 299.1018. Found: 299.1023. Anal. ($C_{14}H_{13}N_5O_3$) C, N, H.

Example 14: ***O*^{6}-Benzyl-2-fluorohypoxanthine (4c)**

O^6-Benzylguanine (1.21 g, 5 mmol) was added to 100 mL of 48% fluoboric acid at -20 °C. Sodium nitrite (1.23 g, 35 mmole) was dissolved in water (5 mL) and 2.5 mL of this sodium nitrite solution was added slowly to the cold fluoboric acid solution. The resulting mixture was stirred for 1 h at or below -15 °C. Additional fluoboric acid (25 mL) was added followed by an additional 2.5 mL of the aqueous sodium nitrite solution. After stirring for an additional 1 h below -15 °C, fluoboric acid (25 mL) was again added and stirring was continued for 1 h. The resulting solution was neutralized with saturated aqueous sodium carbonate solution at -20 °C and was allowed to warm to room temperature. A white precipitate that formed was collected by filtration and was washed with water and dried under vacuum to afford crude **4c**: yield, 0.52 g, 43%. An analytical sample was prepared by chromatography on a Sephadex LH-20 column (3 x 80 cm) eluted with methanol/water (1:1) at 1 mL/min. The desired **4c** eluted in fractions 66 - 77: mp 182 - 183 °C (184 - 185 °C; Robins and Robins, *J. Org. Chem.*, *34*, 2160-2163 (1969)); UV (pH 1) λ_{max} 256 nm (ϵ = 1.117 x 10^4); (pH 6.9) 257 (1.078 x 10^4); (pH 13) 264 (1.063 x 10^4); ^{1}H NMR δ 5.60 (s, 2 H, $ArCH_2$), 7.37-7.57 (m, 5 H, ArH), 8.40 (s, 1 H, H-8), 13.60 (s, 1 H, NH, exchanges with D_2O), ^{19}F NMR δ 23.54 downfield from trifluoroacetic acid standard; MS (EI) calcd. *m/z* for $C_{12}H_9FN_4O$ 244.0760, found 244.0756; Anal. ($C_{12}H_9FN_4O$.2/3 H_2O) C, H, N.

Example 15: ***O*^{6}-Benzyl-*N*^{2}-methylguanine (4e)**

Fluoboric acid (48%, 30 mL) was cooled to -20 °C in an dry ice-acetone bath. *O*^{6}-Benzylguanine (0.362 g, 1.5 mmol) was added with stirring. Sodium nitrite (0.369 g, 10.5 mmol) was dissolved in water (1 mL) and 0.5 mL of this

solution was added slowly to the cold fluoboric acid solution. The resulting solution was stirred at or below -15 °C for 1 h. More fluoboric acid (5 mL) was then added followed by 0.5 mL of the sodium nitrite solution. After stirring for 1 h at or below -15 °C, fluoboric acid (5 mL) was again added and stirring was continued for an additional 1 h. Methylamine (40% in water, 60 mL) was then added at -20 °C, and the resulting basic solution was stirred at room temperature for 2 days. The solvent was evaporated under reduced pressure to produce a white solid. The solid was suspended in 50 mL of H_2O with stirring for 10 min. Undissolved material was collected by filtration and washed with water. This solid was dissolved in 40 mL methanol/water (1:1) to which was added 1.2 mL of 28% aqueous ammonia solution. The solution was loaded on a 3 x 80 cm Sephadex LH-20 column eluted with $MeOH/H_2O/NH_4OH$ (30:70:3) at 1 mL/min. Column eluent was continuously monitored at 280 nm and fractions (10 mL) were collected. Evaporation of the pooled fractions 106-127 gave an analytically pure sample of **4e**: yield, 85 mg (22%); mp 189 - 190 °C; UV (pH 1) λ_{max} 238 nm (sh) (ϵ = 0.665 x 10^4), 297 (0.904 x 10^4); (pH 6.9) 246 (0.898 x 10^4), 290 (0.676 x 10^4); (pH 13) 240 (sh) (0.615 x 10^4), 294 (0.674 x 10^4); ^1H NMR δ 2.30 (d, 3 H, CH_3), 5.50 (s, 2 H, $ArCH_2$), 6.75 (m, 1 H, MeNH, exchanges with D_2O), 7.31-7.53 (m, 5 H, ArH), 7.82 (s, 1 H, H-8), 12.53 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. *m/z* for $C_{13}H_{13}N_5O$ 255.1120, found 255.1107; Anal. ($C_{13}H_{13}N_5O.1/2\ H_2O$) C, H, N.

<u>Example 16:</u> **O^6-Benzyl-N^2,N^2-dimethylguanine (4f)**

Fluoboric acid (48%, 40 mL) was cooled to -20 °C in an dry ice-acetone bath. O^6-Benzylguanine (0.482 g, 2.0 mmol) was added with stirring. Sodium nitrite (0.492 g, 14.0 mmol) was dissolved in water (2 mL) and 1 mL of this solution was added slowly to the cold fluoboric acid solution. The resulting solution was stirred at or below -15 °C for 1 h. More fluoboric acid (10 mL) was added

followed by the addition of 1 mL of the sodium nitrite solution. After stirring for 1 h at or below -15 °C, additional fluoboric acid (10 mL) was added with stirring for 1 h. Dimethylamine (40% in water, 60 mL) was then added to the solution at -20 °C, and the resulting mixture was allowed to warm to room temperature. The suspension became a clear solution and a precipitate formed within 10 min. After standing overnight at room temperature the precipitate was collected by filtration and was washed with water. The solid was crystallized from 50% aqueous ethanol to give an analytically pure sample of **4f**: yield, 0.25 g (46%); mp 220 - 221 °C dec.; UV (pH 1) λ_{max} 248 nm (sh) (ϵ = 0.512 x 10^4), 303 (0.908 x 10^4); (pH 6.9) 251 (1.152 x 10^4), 299 (0.686 x 10^4); (pH 13) 248 (sh) (0.766 x 10^4), 299 (0.710 x 10^4); ^{1}H NMR δ 3.12 (s, 6 H, CH_3), 5.54 (s, 2 H, $ArCH_2$), 7.36-7.51 (m, 5 H, ArH), 7.84 (s, 1 H, H-8), 12.56 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. *m/z* for $C_{14}H_{15}N_5O$ 269.1276, found 269.1254; Anal. ($C_{14}H_{15}N_5O$) C, H, N.

<u>Example 17</u>: **2,4-Diamino-6-benzyloxy-5-bromopyrimidine (3f)**

2,4-Diamino-5-bromo-6-chloropyrimidine (Phillips et. al., *J. Org. Chem.*, *29*, 1488 - 1490 (1963)) (2.3 g, 10 mmol) was added to a solution of sodium (0.29 g, 12.5 mmol) in benzyl alcohol (10 mL) under argon. The solution was heated in a 130 °C oil bath for 3 h and the benzyl alcohol was evaporated under reduced pressure to give a white solid. This solid was washed with water, and air dried. Crystallization from 50% aqueous ethanol gave white crystalline needles of **3f**: yield, 2.32 g (76%); mp 165 - 166 °C (lit. 136 °C; Kosary et. al., *Acta Pharm. Hung.*, 49, 241 - 247 (1989)); UV (pH 1) λ_{max} 236 nm (ϵ = 0.873 x 10^4), 291 (1.388 x 10^4); (pH 6.9) 236 (0.850 x 10^4), 277 (0.835 x 10^4); (pH 13) 234 (0.869 x 10^4), 277 (0.827 x 10^4); ^{1}H NMR δ 5.30 (s, 2 H, $ArCH_2$), 6.15 (s, 2 H, NH_2, exchange with D_2O), 6.32 (s, 2 H, NH_2, exchange with D_2O), 7.31 - 7.45 (m, 5 H, ArH); MS (EI) calcd. *m/z* for $C_{11}H_{11}N_4O^{79}Br$ 294.0115,

found 294.0127; calcd. *m/z* for $C_{11}H_{11}N_4O^{81}Br$ 296.0094, found 296.0083; Anal. ($C_{11}H_{11}N_4OBr$) C, H, N.

Example 18: **2-Amino-4-chloro-5-nitropyrimidine**

A suspension of 2-amino-4-hydroxy-5-nitropyrimidine (5.0 g, 32.1 mmol) in phosphorous oxychloride (100 mL) was refluxed overnight, and the excess phosphorous oxychloride was evaporated under reduced pressure. The residue was mixed with ice (100 g) in an ice-bath, and the mixture was neutralized with concentrated aqueous sodium carbonate solution. A yellow precipitate was collected by filtration and washed with water: yield, 1.39 g (25%); mp 191 - 194 °C dec.; ^1H NMR δ 8.45 (br s, 2 H, NH_2, exchange with D_2O), 9.03 (s, 1 H, H-6); MS (EI) calcd. *m/z* for $C_4H_3N_4O_2{}^{35}Cl$ 173.9944, found 173.9934; calcd. *m/z* for $C_4H_3N_4O_2{}^{37}Cl$ 175.9915, found 175.9916.

Example 19: **2-Amino-4-benzyloxy-5-nitropyrimidine (5a)**

2-Amino-4-chloro-5-nitropyrimidine (0.70 g, 4.0 mmol) was added to a solution of sodium (0.12 g, 5.2 mmol) in benzyl alcohol (8 mL) under argon. The solution was heated in a 130 °C oil bath for 3 h, and approximately half of the benzyl alcohol was evaporated under reduced pressure. The residue was poured into water (50 mL) with constant stirring for 10 min. After neutralization with glacial acetic acid, a brown precipitate formed which was collected by filtration and washed with water. This solid was crystallized from benzene to give **5a** as a golden crystalline solid: yield, 126 mg (13%); mp 164 - 167 °C; UV (pH 1) λ_{max} 262 nm (ϵ = 0.879 x 10^4), 295 (sh) (0.571 x 10^4); (pH 6.9) 235 (sh) (0.448 x 10^4), 273 (0.360 x 10^4), 326 (1.085 x 10^4); (pH 13) 273 (0.404 x 10^4), 327 (1.055 x 10^4); ^1H NMR δ 5.51 (s, 2 H, $ArCH_2$), 7.35 - 7.54 (m, 5 H, ArH), 8.05 (d, 2 H, NH_2, exchange with D_2O), 8.92 (s, 1 H, H-6); MS (EI) calcd. *m/z* for $C_{11}H_{10}N_4O_3$ 246.0752, found 246.0758; Anal. ($C_{11}H_{10}N_4O_3$) C, H, N.

Example 20: **2-Amino-4-benzyloxy-6-methyl-5-nitropyrimidine (5b)**

2-Amino-4-chloro-6-methyl-5-nitropyrimidine (Boon et al., *J. Chem. Soc.*, 96 - 102 (1951)) (1.24 g, 6.58 mmol) was added to a solution of sodium (0.21 g, 9.13 mmol) in benzyl alcohol (14 mL) under argon. The solution was heated in a 135 °C oil bath for 3.5 h, and was poured into water (70 mL) with constant stirring for 10 min. After neutralization with glacial acetic acid, a yellow precipitate formed which was collected by filtration and washed with water. This solid was crystallized from benzene to give **5b** as a bright yellow crystalline solid: yield, 0.57 g (33%); mp 159 - 160 °C; UV (pH 1) λ_{max} 268 nm (ϵ = 0.783 x 10^4), 345 (sh) (0.104 x 10^4); (pH 6.9) 282 (0.564 x 10^4), 345 (sh) (0.338 x 10^4); (pH 13) 282 (0.549 x 10^4), 345 (sh) (0.332 x 10^4); ^{1}H NMR δ 2.35 (s, 3 H, CH_3), 5.44 (s, 2 H, $ArCH_2$), 7.34 - 7.46 (m, 5 H, ArH), 7.64 (b s, 2 H, NH_2, exchange with D_2O); MS (EI) calcd. *m/z* for $C_{12}H_{12}N_4O_3$ 260.0908, found 260.0913; Anal.($C_{12}H_{12}N_4O_3$) C, H, N.

Example 21: **2,4-Diamino-6-benzyloxy-*s*-triazine (6)**

2,4-Diamino-6-chloro-*s*-triazine (2.25 g, 15.0 mmol) was added to a solution of sodium (0.43 g, 18.8 mmol) in benzyl alcohol (30 mL) under argon. The suspension was heated in a 130 °C oil bath for 3.5 h. The excess benzyl alcohol was removed under vacuum and the resulting solid was collected with the aid of benzene, and washed with water (100 mL): yield, 1.83 g (56%); mp 184 - 185 °C (lit. 186 - 188 °C; Wakabayashi et al., *Nippon Dojo-Hiryogaku Zasshi*, *41*, 193 - 200 (1970)); UV (pH 1) λ_{max} 233 nm (sh) (ϵ = 0.589 x 10^4); (pH 6.9) 238 (sh) (0.111 x 10^4); (pH 13) 240 (sh) (0.073 x 10^4); ^{1}H NMR δ 5.25 (s, 2 H, $ArCH_2$), 6.63 (s, 4 H, NH_2, exchange with D_2O), 7.30 - 7.42 (m, 5 H, ArH); MS (EI) calcd. *m/z* for $C_{10}H_{11}N_5O$ 217.0963, found 217.0955.

Example 22: **2-Amino-6-chloro-8-trifluoromethylpurine**

A suspension of 8-trifluoromethylguanine (Pfleiderer and Shanshal, *Liebigs Ann. Chem.*, *726*, 201 - 215 (1969)) (2.0 g, 9.1 mmol) in phosphorous oxychloride (20 mL) was refluxed for 3 h. Excess phosphorous oxychloride was evaporated under reduced pressure. The resulting residue was mixed with ice-water (100 g), and the pH was adjusted to 3 - 4 with a concentrated aqueous NaOH solution. The resulting solution was mixed with MeOH (100 mL) and approximately half (i.e., 100 mL) of the aqueous methanol solution was loaded on a 3 x 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm and fractions (10 mL) were collected. The remainder of the reaction mixture in MeOH/H_2O was chromatographed separately under identical conditions. The desired product eluted in fractions 73 - 85. Evaporation of solvent from the pooled fractions 73 - 85 from both chromatographic runs afforded analytically pure 2-amino-6-chloro-8-trifluoromethylpurine: yield, 0.94 g (43%); mp >225 °C dec.; UV (pH 1) λ_{max} 245 nm (ϵ = 0.501 x 10^4), 314 (0.746 x x 10^4); (pH 6.9) 270 (0.265 x 10^4), 315 (0.612 x 10^4); (pH 13) 272 (0.269 x 10^4), 314 (0.612 x 10^4); ^{1}H NMR δ 7.19 (s, 2 H, NH_2, exchange with D_2O), 14.25 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. *m/z* for $C_6H_3N_5F_3{}^{35}Cl$ 237.0029, found 237.0011; calcd. *m/z* for $C_6H_3N_5F_3{}^{37}Cl$ 239.0000, found 238.9987; Anal. ($C_6H_3N_5F_3C$) C, H, N, F, Cl.

Example 23: **O^6-Benzyl-8-trifluoromethylguanine (1e)**

Sodium (0.10 g, 4.3 mmol) was stirred in 5 mL of benzyl alcohol until all had reacted. 2-Amino-6-chloro-8-trifluoromethylpurine (0.475 g, 2.0 mmol) was added, and the reaction mixture was heated in a 135 °C oil bath for 3.5 h. The benzyl alcohol was removed by vacuum distillation yielding a brown oil. The oil was dissolved in water (50 mL) and was acidified with glacial acetic acid to produce a pale yellow precipitate. The precipitate was

collected by filtration and washed with water. The crude product was loaded on a 2.5 x 35 cm silica gel column (Davisil grade 633, 200 - 425 mesh, 60 Å). Elution was carried out with 5% EtOH in $CHCl_3$ to provide analytically pure O^6-benzyl-8-trifluoromethylguanine (**1e**): yield, 0.42 g (67%); mp 214 - 216 °C dec.; UV (pH 1) λ_{max} 291 nm (ϵ = 1.229 x 10^4); (pH 6.9) 244 (0.470 x 10^4), 289 (1.023 x 10^4); (pH 13) 247 (sh) (0.393 x 10^4), 290 (0.923 x 10^4); ^{1}H NMR δ 5.51 (s, 2 H, $ArCH_2$), 6.82 (s, 2 H, NH_2, exchange with D_2O), 7.38 - 7.55 (m, 5 H, ArH), 13.75 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd. *m/z* for $C_{13}H_{10}N_5OF_3$ 309.0837, found 309.0827; Anal. ($C_{13}H_{10}N_5OF_3$) C, H, N, F.

<u>Example 24</u>: **O^6-Benzyl-8-trifluoromethyl-9-methylguanine (7)**

To O^6-benzyl-8-trifluoromethylguanine (**1e**) (200 mg, 0.65 mmol) under argon was added 0.66 mL of a 1.0 M solution of sodium ethoxide in ethanol. The solution was stirred for 10 min and the ethanol was removed under vacuum. The remaining solid was dissolved in anhydrous DMF (1.5 mL), and methyl iodide (49 μL, 0.78 mmol) was added to the solution. This solution was stirred at room temperature for 1 h, and 1.5 mL additional DMF was added. The solution was stirred at room temperature overnight. The solvent was evaporated under reduced pressure. The crude solid was loaded on a 2.5 x 35 cm silica gel column (Davisil grade 633, 200 - 425 mesh, 60 Å). Elution was carried out with chloroform/hexane (3:1) to provide analytically pure O^6-benzyl-8-trifluoromethyl-9-methylguanine (**7**): yield, 95 mg (45%); mp 86 - 89 °C; UV (pH 1) λ_{max} 244 nm (ϵ = 0.581 x 10^4), 286 (1.274 x 10^4); (pH 6.9) 252 (0.608 x 10^4), 288 (1.022 x 10^4); (pH 13) 252 (0.618 x 10^4), 288 (1.038 x 10^4); ^{1}H NMR δ 3.70 (s, 3 H, CH_3), 5.51 (s, 2 H, $ArCH_2$), 6.91 (s, 2 H, NH_2, exchange with D_2O), 7.38 - 7.54 (m, 5 H, ArH); MS (EI) calcd. *m/z* for $C_{14}H_{12}N_5OF_3$ 323.0994, found 323.0978; Anal. ($C_{14}H_{12}N_5OF_3$) C, H, N, F.

Example 25: **8-Aza-O^6-benzyl-9-methylguanin (8a)**

8-Aza-O^6-benzylguanine (0.484 g, 2.0 mmol) was mixed with 4 mL of 0.5 M sodium ethoxide in ethanol and stirred for 30 min. The ethanol was evaporated under reduced pressure. The residue was dissolved in anhydrous DMF (6 mL), and methyl iodide (0.15 mL, 2.4 mmol) was added. The clear solution became cloudy within 10 min, and the resulting mixture was stirred overnight at room temperature. DMF was evaporated under reduced pressure to give a brown solid. The solid was dissolved in chloroform and loaded on a silica gel column (Davisil grade 633, 200-425 mesh, 60 Å). Product **8a** was eluted with chloroform; yield, 138 mg (27%); mp 178-179 °C; UV (pH 1) λ_{max} 243 nm (sh) (ϵ = 0.556 x 10^4), 284 (1.112 x 10^4); (pH 6.9) 243 (0.553 x 10^4), 290 (0.998 x 10^4); (pH 13) 242 (0.549 x 10^4), 290 (1.010 x 10^4); ^{1}H NMR δ 3.96 (s, 3 H, CH_3), 5.57 (s, 2 H, $ArCH_2$), 7.18 (s, 2 H, NH_2, exchange with D_2O), 7.38-7.57 (m, 5 H, ArH); MS (EI) calcd m/z for $C_{12}H_{12}N_6O$ 256.1072, found 256.1086; Anal. ($C_{12}H_{12}N_6O$) C, H, N.

Example 26: **8-Aza-O^6-benzyl-9-(pivaloyloxymethyl)guanine (8b) and 8-Aza-O^6-benzyl-7-(pivaloyloxymethyl)guanine (9)**

8-Aza-O^6-benzylguanine (0.484 g, 2.0 mmol) was mixed with 4 mL of 0.5 M sodium ethoxide in ethanol and stirred for 30 min. The ethanol was evaporated under reduced pressure. The residue was dissolved in anhydrous DMF (6 mL), and chloromethyl pivalate (0.3 mL, 2.1 mmol) was added. The clear solution was stirred for 8 h at room temperature. DMF was evaporated under reduced pressure to give a brown solid. The solid was dissolved in chloroform and loaded on a silica gel column (Davisil grade 633, 200 - 425 mesh, 60 Å). The 9-isomer (**8b**) was eluted from the column with $CHCl_3$:hexane (4:1) while the 7-isomer (**9**) was subsequently eluted with $CHCl_3$. 8-Aza-O^6-benzyl-9-(pivaloyloxymethyl)guanine (**8b**): yield, 405 mg (57%); mp 119-120 °C; UV (pH 1) λ_{max} 246 nm (ϵ = 0.494 x 10^4), 286 (0.878 x 10^4); (pH 6.9) 247 (0.472 x 10^4), 288 (0.819 x

10^4); (pH 13) (decomposes to 8-aza-O^6-benzylguanine); ^1H NMR δ 1.10 (s, 9 H, $C(CH_3)_3$), 5.50 (s, 2 H, $ArCH_2$), 6.31 (s, 2 H, CH_2), 7.38 (s, 2 H, NH_2, exchange with D_2O), 7.40-7.54 (m, 5 H, ArH); MS (EI) calcd m/z for $C_{17}H_{20}N_6O_3$ 356.1596, found 356.1578; Anal. ($C_{17}H_{20}N_6O_3.1/5H_2O$) C, H, N. 8-Aza-$O^6$-benzyl-7-(pivaloyloxymethyl)guanine (9): yield, 103 mg (15%); mp 153-154 °C; UV (pH 1) λ_{max} 244 nm (ϵ = 0.820 x 10^4), 294 (1.249 x 10^4); (pH 6.9) 250 (sh) (0.296 x 10^4), 313 (0.503 x 10^4); (pH 13) (decomposes to 8-aza-O^6-benzylguanine); ^1H NMR δ 1.12 (s, 9 H, $C(CH_3)_3$), 5.56 (s, 2 H, $ArCH_2$), 6.40 (s, 2 H, CH_2), 7.04 (s, 2 H, NH_2, exchange with D_2O), 7.4-7.58 (m, 5 H, ArH); MS (EI) calcd m/z for $C_{17}H_{20}N_6O_3$ 356.1596, found 356.1602; Anal. ($C_{17}H_{20}N_6O_3$).

<u>Example 27</u>: **O^6-Benzyl-8-bromo-9-methylguanine (10a)**

O^6-Benzyl-9-methylguanine (0.252 g, 1.0 mmol) and sodium bicarbonate (0.084 g, 1.0 mmol) were dissolved in anhydrous DMF (2 mL) under argon. Bromine (52 μL, 1.0 mmol) was added to the solution and the resulting mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in chloroform, and loaded on a silica gel column (Davisil grade 633, 200 - 425 mesh, 60 Å). Product **10a** was eluted with chloroform; yield, 180 mg (52%); mp 150-152 °C; UV (pH1) λ_{max} 248 nm (ϵ = 0.753 x 10^4), 292 (1.398 x 10^4); (pH 6.9) 251 (0.919 x 10^4), 287 (1.306 x 10^4); (pH 13) 251 (0.906 x 10^4), 287 (1.296 x 10^4); ^1H NMR δ 3.53 (s, 3 H, CH_3), 5.47 (s, 2 H, $ArCH_2$), 6.61 (s, 2 H, NH_2, exchange with D_2O), 7.35-7.52 (m, 5 H, ArH); MS (EI) calcd m/z for $C_{13}H_{12}N_5O^{79}Br$ 333.0225, found 333.0228; calcd m/z for $C_{13}H_{12}N_5O^{81}Br$ 335.0205, found 335.0188; Anal. ($C_{13}H_{12}N_5OBr$) C, H, N, Br.

<u>Example 28</u>: **O^6-Benzyl-8-bromo-9-(pivaloyloxymethyl)guanine (10b) and O^6-benzyl-8-bromo-7-(pivaloyloxymethyl)guanine (11)**

O^6-Benzyl-8-bromoguanine (Chae et al., *J. Med. Chem.*, *38*, 342-347 (1995)) (0.48 g, 1.5 mmol) was mixed with 1.5 mL of a 1.0 M solution of sodium ethoxide in ethanol and was stirred for 20 min. The ethanol was removed under reduced pressure and the solid residue was dissolved in DMF (5 mL). Chloromethylpivalate (0.24 mL, 1.65 mmol) was then added and the solution was stirred overnight. The DMF was removed under reduced pressure. The residue was dissolved in chloroform and was loaded on a silica gel column (Davisil grade 633, 200-425 mesh, 60Å) eluted with chloroform. The 9-isomer (**10b**) eluted earlier than the 7-isomer with chloroform and **10b** was recovered in pure form under these conditions. O^6-Benzyl-8-bromo-9-(pivaloyloxymethyl)guanine (**10b**): yield, 150 mg (23%); mp 217-218°C; UV (pH 1) λ_{max} 250 nm (ϵ = 0.944 x 10^4), 291 (1.166 x 10^4); (pH 6.9) 266 (0.916 x 10^4), 295 (0.916 x 10^4); (pH 13) decomposes to O^6-benzyl-8-bromoguanine; ^1H NMR δ 1.13 (s, 9H, $C(CH_3)_3$), 5.48 (s, 2H, $ArCH_2$), 5.93 (s, 2H, CH_2), 6.80 (s, 2H, NH_2, exchange with D_2O), 7.35-7.52 (m, 5H, ArH). MS (EI) calcd *m/z* for $C_{18}H_{20}N_5O_3{}^{79}Br$ 433.0750, found 433.0725; calcd m/z for $C_{18}H_{20}N_5O_3{}^{81}Br$ 435.0729, found 435.0672; Anal. ($C_{18}H_{20}N_5O_3Br$) C, H, N, Br. The recovered 7-isomer (**11**) was rechromatographed on a silica gel column (Davisil grade 633, 200-425 mesh, 60 Å) which was eluted first with $CHCl_3$/hexane (1:1) followed by $CHCl_3$ to recover O^6-benzyl-8-bromo-7-(pivaloyloxymethyl)guanine (**11**).

<u>Example 29</u>: **O^6-Benzyl-7-(pivaloyloxymethyl)guanine (12)**

O^6-Benzylguanine (2.41 g, 10 mmol) was mixed with 10 mL of a 1.0 M solution of sodium ethoxide in ethanol and was stirred for 30 min. The ethanol was evaporated under reduced pressure. The residue was dissolved in anhydrous DMF (30 mL), and chloromethyl- pivalate (Aldrich) (1.5 mL,

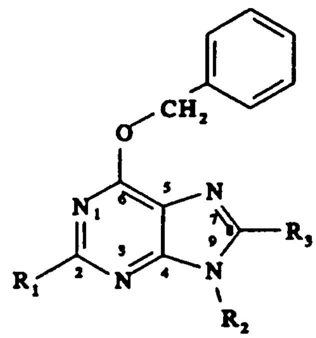
10.4 mmol) was added. The clear solution was stirred overnight at room temperature. DMF was evaporated under reduced pressure to give a pale peach-colored solid. The solid was dissolved in chloroform/ethanol (9:1) and loaded on a silica gel column (Davisil grade 633, 200 - 425 mesh, 60 Å). The column was eluted with chloroform/ethanol (9:1) to elute the 9-isomer (Chae et al., *J. Med. Chem.*, 37, 342-347 (1994)) followed by the 7-isomer. The 7-isomer (**12**) was further purified by silica gel column chromatography (Davisil grade 633, 200 - 425 mesh, 60 Å) using chloroform/ethanol (98:2) as eluent: yield, 36 mg (1%); mp 166-168 °C dec; UV (pH 1) λ_{max} 240 nm (sh) (ϵ = 0.656 x 10^4), 290 (1.164 x 10^4); (pH 6.9) 240 (sh) (0.635 x 10^4), 293 (0.528 x 10^4); (pH 13) decomposes to O^6-benzylguanine; ^{1}H NMR δ 0.98 (s, 9 H, $C(CH_3)_3$), 5.51 (s, 2 H, $ArCH_2$), 6.07 (s, 2 H, CH_2), 6.32 (s, 2 H, NH_2, exchange with D_2O), 7.36-7.58 (m, 5 H, ArH), 8.25 (s, 1 H, H-8); MS (EI) calcd *m/z* for $C_{18}H_{21}N_5O_3$ 355.1644, found 355.1626.

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A compound of the formula



wherein R_1 is a substituent selected from the group consisting of amino, hydroxy, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, and C_1-C_4 acylamino, R_2 is a substituent selected from the group consisting of hydrogen, C_1-C_4 alkyl, C_1-C_4 aminoalkyl, C_1-C_4 hydroxyalkyl, C_1-C_4 alkylamino C_1-C_4 alkyl, C_1-C_4 dialkylamino alkyl, C_1-C_4 cyanoalkyl, C_1-C_4 carbamoylalkyl, C_1-C_4 pivaloylalkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 carboalkoxyalkyl, ribose, 2'-deoxyribose, the conjugate acid form of a C_1-C_4 carboxyalkyl, and the carboxylate anion of a C_1-C_4 carboxyalkyl as the sodium salt, and R_3 is a substituent selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, thiol, C_1-C_4 alkylthio, trifluoromethylthio, C_1-C_4 thioacyl, hydroxy, C_1-C_4 alkoxy, trifluoromethoxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, C_1-C_4 acyloxy, amino, C_1-C_4 aminoalkyl, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, trifluoromethylamino, ditrifluoromethylamino, aminomethanesulfonyl, amino C_1-C_4 alkylcarbonyl, aminotrifluoromethylcarbonyl, formylamino, nitro, nitroso, C_1-C_4 alkyldiazo, C_5-C_6 aryldiazo, trifluoromethyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, cyano, C_1-C_4 alkyloxycarbonyl, C_1-C_4 alkylcarbonyl, phenyl, phenylcarbonyl, formyl, C_1-C_4 alkoxymethyl, phenoxymethyl, C_2-C_4 vinyl, C_2-C_4 ethynyl, and SO_nR' wherein n is 0, 1, 2, or 3 and R' is hydrogen, C_1-C_4 alkyl, amino, or phenyl, with the proviso that R_1 is not

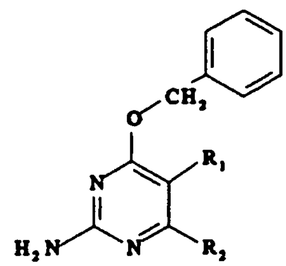
amino when both R_2 and R_3 are hydrogen, and with the proviso that R_1 is not amino or methylamino when R_2 is ribose or 2'-deoxyribose and R_3 is hydrogen.

2. The compound of claim 1, wherein R_1 is selected from the group consisting of amino, hydroxy, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, and C_1-C_4 alkylcarbonylamino, R_2 is selected from the group consisting of hydrogen, C_1-C_4 alkyl, and C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, and R_3 is selected from the group consisting of amino, halo, C_1-C_4 alkyl, hydroxy, and trifluoromethyl.

3. The compound of claim 2, wherein R_1 is selected from the group consisting of amino, hydroxy, methylamino, dimethylamino, and acetylamino, R_2 is selected from the group consisting of hydrogen, methyl, and pivaloyloxymethyl, and R_3 is selected from the group consisting of amino, bromo, methyl, hydroxy, and trifluoromethyl.

4. The compound of claim 3, wherein said compound is selected from the group consisting of 8-amino-O^6-benzylguanine, 8-methyl-O^6-benzylguanine, 8-hydroxy-O^6-benzylguanine, 8-bromo-O^6-benzylguanine, 8-trifluoromethyl-O^6-benzylguanine, O^6-benzylxanthine, O^6-benzyluric acid, N^2-acetyl-O^6-benzyl-8-oxoguanine, O^6-benzyl-N^2-methylguanine, O^6-benzyl-N^2,N^2-dimethylguanine, O^6-benzyl-8-trifluoromethyl-9-methylguanine, O^6-benzyl-8-bromo-9-methylguanine, and O^6-benzyl-8-bromo-9-(pivaloyloxymethyl)-guanine.

5. A compound of the formula

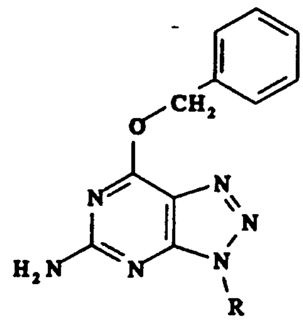


wherein R_1 is NO_2 or NO, and R_2 is a substituent selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, thiol, C_1-C_4 alkylthio, trifluoromethylthio, C_1-C_4 thioacyl, hydroxy, C_1-C_4 alkoxy, trifluoromethoxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, C_1-C_4 acyloxy, C_1-C_4 aminoalkyl, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, trifluoromethylamino, ditrifluoromethylamino, aminomethanesulfonyl, amino C_1-C_4 alkylcarbonyl, aminotrifluoromethylcarbonyl, formylamino, nitro, nitroso, C_1-C_4 alkyldiazo, C_5-C_6 aryldiazo, trifluoromethyl, C_1-C_4 haloalkyl, C_1-C_4 cyanoalkyl, cyano, C_1-C_4 alkyloxycarbonyl, C_1-C_4 alkylcarbonyl, phenyl, phenylcarbonyl, formyl, C_1-C_4 alkoxymethyl, phenoxymethyl, C_2-C_4 vinyl, C_2-C_4 ethynyl, and SO_nR' wherein n is 0, 1, 2, or 3 and R' is hydrogen, C_1-C_4 alkyl, amino, or phenyl.

6. The compound of claim 5, wherein R_1 is NO_2, and R_2 is selected from the group consisting of hydrogen and C_1-C_4 alkyl.

7. The compound of claim 6, wherein said compound is seleced from the group consisting of 2-amino-4-benzyloxy-5-nitropyrimidine and 2-amino-4-benzyloxy-6-methyl-5-nitropyrimidine.

8. A compound of the formula

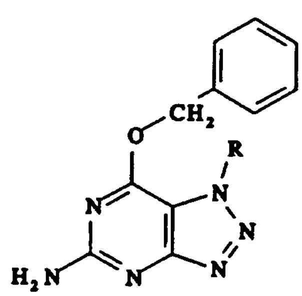


wherein R is selected from the group consisting of C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl.

9. The compound of claim 8, wherein R is selected from the group consisting of C_1-C_4 alkyl and C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl.

10. The compound of claim 9, wherein said compound is selected from the group consisting of 8-aza-O^6-benzyl-9-methylguanine and 8-aza-O^6-benzyl-9-(pivaloyloxymethyl) guanine.

11. A compound of the formula



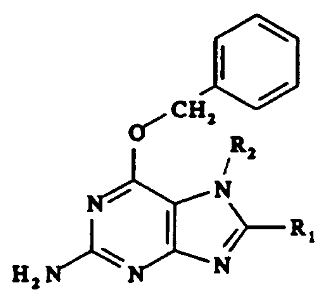
wherein said R is selected from the group consisting of C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano

C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl.

12. The compound of claim 11, wherein said R is C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl.

13. The compound of claim 12, wherein said compound is 8-aza-O^6-benzyl-7-(pivaloyloxymethyl)guanine.

14. A compound of the formula



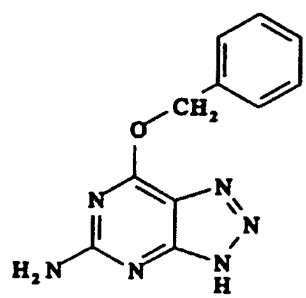
wherein R_1 is selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, halo C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl, and R_2 is selected from the group consisting of C_1-C_4 alkyl, halo C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_1-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_1-C_4 alkyl, cyano C_1-C_4 alkyl, aminocarbonyl C_1-C_4 alkyl, hydroxy C_1-C_4 alkyl, and C_1-C_4 alkyloxy C_1-C_4 alkyl, with the proviso that when R_1 is hydrogen, R_2 is selected from the group consisting of halo C_1-C_4 alkyl, C_1-C_4 alkyloxy C_1-C_4 alkyl, C_1-C_6 alkylcarbonyloxy C_1-C_4 alkyl, C_3-C_4 alkyloxycarbonyl C_1-C_4 alkyl, carboxy C_2-C_4 alkyl, cyano C_2-C_4 alkyl, aminocarbonyl C_2-C_4 alkyl, and hydroxy C_1-C_3 alkyl.

15. The compound of claim 14, wherein said compound is selected from the group consisting of O^6-benzyl-8-bromo-7-(pivaloyloxymethyl)guanine and O^6-benzyl-7-(pivaloyloxymethyl)guanine.

16. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and at least one compound of any of claims 1-15.

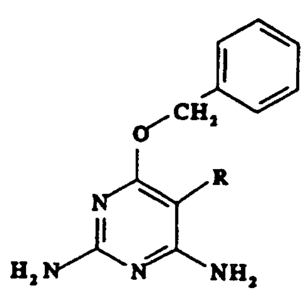
17. The pharmaceutical composition of claim 16, wherein said pharmaceutically acceptable carrier comprises polyethylene glycol.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula



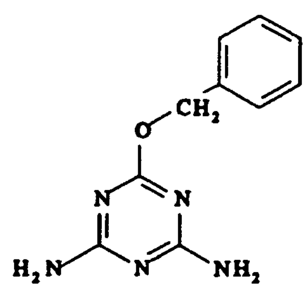
wherein said pharmaceutically acceptable carrier comprises polyethylene glycol.

19. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula



wherein R is a substituent selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, thiol, C_1-C_4 alkylthio, trifluoromethylthio, C_1-C_4 thioacyl, hydroxy, C_1-C_4 alkoxy, trifluoromethoxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, C_1-C_4 acyloxy, amino, C_1-C_4 aminoalkyl, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, trifluoromethylamino, ditrifluoromethylamino, aminomethanesulfonyl, amino C_1-C_4 alkylcarbonyl, aminotrifluoromethylcarbonyl, formylamino, nitro, nitroso, C_1-C_4 alkyldiazo, C_5-C_6 aryldiazo, trifluoromethyl, C_1-C_4 haloalkyl, cyanomethyl, C_1-C_4 cyanoalkyl, cyano, C_1-C_4 alkyloxycarbonyl, C_1-C_4 alkylcarbonyl, phenyl, phenylcarbonyl, C_1-C_4 acyl, formyl, C_1-C_4 alkoxymethyl, phenoxymethyl, C_2-C_4 vinyl, C_2-C_4 ethynyl, and SO_nR_1 wherein n is 0, 1, 2, or 3 and R_1 is hydrogen, C_1-C_4 alkyl, amino, or phenyl, and wherein said pharmaceutically acceptable carrier comprises polyethylene glycol.

20. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of the formula



wherein said pharmaceutically acceptable carrier comprises polyethylene glycol.

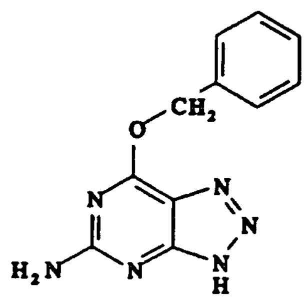
21. A method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent that causes cytotoxic lesions at the O^6-position of guanine, which method comprises:

administering to a mammal an effective amount of a compound of any of claims 1-15, and

administering to said mammal an effective amount of an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine.

22. A method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine, which method comprises:

administering to a mammal an effective amount of a compound of the formula

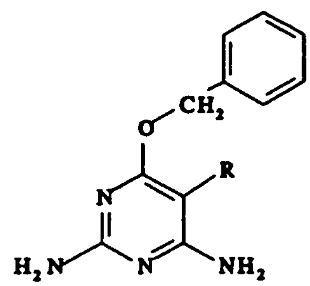


and

administering to said mammal an effective amount of an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine.

23. A method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent that causes cytotoxic lesions at the O^6-position of guanine, which method comprises:

administering to a mammal an effective amount of a compound of the formula

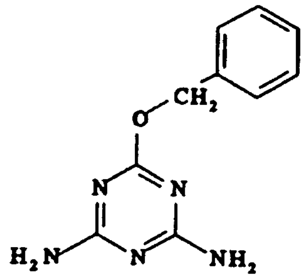


wherein R is a substituent selected from the group consisting of hydrogen, halo, C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, thiol, C_1-C_4 alkylthio, trifluoromethylthio, C_1-C_4 thioacyl, hydroxy, C_1-C_4 alkoxy, trifluoromethoxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, C_1-C_4 acyloxy, amino, C_1-C_4 aminoalkyl, C_1-C_4 alkylamino, C_1-C_4 dialkylamino, trifluoromethylamino, ditrifluoromethylamino, aminomethanesulfonyl, amino C_1-C_4 alkylcarbonyl, aminotrifluoromethylcarbonyl, formylamino, nitro, nitroso, C_1-C_4 alkyldiazo, C_5-C_6 aryldiazo, trifluoromethyl, C_1-C_4 haloalkyl, cyanomethyl, C_1-C_4 cyanoalkyl, cyano, C_1-C_4 alkyloxycarbonyl, C_1-C_4 alkylcarbonyl, phenyl, phenylcarbonyl, C_1-C_4 acyl, formyl, C_1-C_4 alkoxymethyl, phenoxymethyl, C_2-C_4 vinyl, C_2-C_4 ethynyl, and SO_nR_1 wherein n is 0, 1, 2, or 3 and R_1 is hydrogen, C_1-C_4 alkyl, amino, or phenyl, and

administering to said mammal an effective amount of an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine.

24. A method of enhancing the chemotherapeutic treatment of tumor cells in a mammal with an antineoplastic alkylating agent that causes cytotoxic lesions at the O^6-position of guanine, which method comprises:

administering to a mammal an effective amount of a compound of the formula



and

administering to said mammal an effective amount of an antineoplastic alkylating agent which causes cytotoxic lesions at the O^6-position of guanine.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/09702

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D473/18 C07D487/04 C07D473/06 C07D473/40 C07D239/46
C07D239/48 C07D239/50 C07D251/52 A61K31/52 A61K31/505
//(C07D487/04,249:00,239:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 101, no. 25, 17 December 1984, Columbus, Ohio, US; abstract no. 230466q, page 765 ;column R ; see abstract & HETEROCYCLES, vol.22, no.8, 1984 pages 1789 - 1790 RAM SIYA ET AL ---	19
X	THE JOURNAL OF ORGANIC CHEMISTRY, vol.34, no.7, July 1969 pages 2160 - 2163 MORRIS J. ROBINS ET AL *Page 2161: column 2: formula 13* ---	19
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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
8 November 1995	16.11.1995

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Luyten, H

INTERNATIONAL SEARCH REPORT

Intern' al Application No
PCT/US 95/09702

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS SERVICE REGISTRY HANDBOOK . NUMBER SECTION 1965-1971 *Page 11267r: column 3, registry number 30360-74-8* ---	20
P,X	JOURNAL OF MEDICINAL CHEMISTRY, vol.38, no.2, 20 January 1995 pages 359 - 365 MI-YOUNG CHAE ET AL ---	5-7
A	JOURNAL OF MEDICINAL CHEMISTRY, vol.37, no.3, 4 February 1994 pages 342 - 347 MI-YOUNG CHAE ET AL cited in the application *Article* ---	1-14
A	JOURNAL OF MEDICINAL CHEMISTRY, vol.35, no.23, 13 November 1992 pages 4486 - 4491 ROBERT C. MOSCHEL ET AL cited in the application *Article* -----	1-14

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09702

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 21-24 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.